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Body Fat Determination of Stock-Type Horses in Varying Body Condition by Carcass Dissection, Rump Fat Thickness, and Deuterium Oxide Dilution and Fatty Acid Composition of Adipose Tissues

Emily Nicole Ferjak

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By

Emily Nicole Ferjak

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By

Emily Nicole Ferjak

Approved:

_____

Clay A. Cavinder
(Major Professor)

_____

Thu T. N. Dinh
(Committee Member)

_____

Derris B. Burnett
(Committee Member)

_____

Chris Mortensen
(Committee Member)

_____

Jamie Larson
(Graduate Coordinator)

_____

John Blanton, Jr.
Department Head

_____

George M. Hopper
Dean
College of Agricultural and Life Sciences

Name: Emily Nicole Ferjak

Date of Degree: August 11, 2017

Institution: Mississippi State University

Major Field: Agriculture

Major Professor: Clay A. Cavinder

Title of Study: Body fat determination of stock-type horses in varying body condition by carcass dissection, rump fat thickness, and deuterium oxide dilution and fatty acid composition of adipose tissues

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Candidate for Degree of Master of Science

The primary objectives of the study were to compare 2 body fat (%, BF) prediction methods for stock-type horses by rump fat thickness (RFT) and D_2O dilution with actual tissue fat analysis by near-infrared spectroscopy (NIR) and to identify the relationships among BF, BCS, and physical measurements. Secondary objectives were to determine the fatty acid (FA) composition of mesenteric (MS), cardiac (CD), subcutaneous (SC), intermuscular (IM), and leaf fat (LF) and to identify relationships between of FA composition and BCS in horses. Results indicated that D_2O dilution is an accurate predictor of BF, and RFT alone does not accurately predict BF. Additionally, BCS may be useful in predicting BF when used with other physical measurements. The effects of BCS and fat depot on FA composition were independent of each other. The more influential factor in FA composition of adipose tissues was fat depot as opposed to BCS.

DEDICATION

I dedicate this thesis first to my savior, Jesus Christ, who has blessed me with the opportunity to further my education and reach so many of my goals. I'd like to thank Him for his faithfulness, guidance, and many answered prayers, without which the completion of this thesis would not have been possible. I would also like to thank my family for their endless love and encouragement over the past two years. Without my parent's selfless dedication to supporting my passion for horses, and teaching me the value of hard work and perseverance, I could not have made it this far. Lastly, I thank my loving fiancé, Ryan Tittle, for the sacrifices he has made to see me through successfully finishing graduate school.

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CHAPTER I

INTRODUCTION

Body condition, or the total amount of fat stored in an animal's body, has a significant impact on various physiological systems; however, many of the exact mechanisms triggering such effects remain unclear. In several livestock species, a positive relationship between body condition and reproductive function is well documented. Moderate body condition has been shown to be positively related to reproductive performance in cattle (Donaldson, 1969; Lamond, 1969) and sheep (Sejian et al., 2009). Similar results have been recorded in horses as increased breeding efficiency has been observed in mares entering the breeding season at a BCS of 5.0 (moderate body condition) or above (Henneke et al., 1984; Kubiak et al., 1987; Cavinder et al., 2009) compared to low body condition. Kubiak et al. (1987) established that mares entering the breeding season with body fat (BF, %) of 11.5-15% require fewer cycles for conception and have higher conception rates than mares entering the breeding season with BF of < 11.5% condition. Additionally, mares maintained at 11.5 - 15% vs < 11.5% BF ovulate sooner (Kubiak et al., 1987). Unlike cattle, mares in high body condition at time of parturition exhibit foaling characteristics similar to mares in moderate body condition (Kubiak et al., 1988), suggesting that maintaining broodmares at a high BCS (7-8) neither impairs nor improves reproductive efficiency. Thus, it is recommended that

mares used for breeding be maintained at a BCS of at least 5.0 (Henneke et al., 1984; Cavinder et al., 2009).

While obesity is a rampant equine metabolic condition in the U.S. (eXtension, 2017), malnutrition is an equally pressing issue due to its severe health effects and many implications, including death (Brinkmann et al., 2013). Largely attributable to the discontinuation of horse slaughter in the United States, the Unwanted Horse Coalition (UHC) and similar groups have observed increased numbers of overly thin, malnourished horses due to a lack of marketing options for owners who can no longer afford feed costs. According to the UHC, it is difficult to precisely determine the number of unwanted horses in the United States; however, it is known that the number exceeds the available resources to provide for them. In a 2009 UHC survey, 63% of equine rescue/retirement facilities reported to be at full capacity, and thus denied 38% of horses presented to them. Currently, there is no defined threshold below which a horse can be categorized as overly thin/undernourished in equine welfare legal cases. Identifying a minimally invasive method of determining overall BF would allow law enforcement officers along with veterinarians to more objectively determine the energy status of horses in neglect/abuse cases, and thus set legal limits and aid in prosecution of offenders.

As mentioned earlier, obesity is a significant issue throughout the equine industry. In the United States, improper nutrition is one of the foremost health issues that plague the horse populace, largely due to flawed management practices. It is common for horse owners to employ improper feeding management strategies, leading to overweight horses, thus causing a range of obesity-related health issues including insulin resistance and inflammatory responses (Vick et al., 2007), as well as an unnecessary expenditure of

economic resources. Cost-effective feeding is especially pertinent to solving the issue of unwanted horses in the United States. Due to the fact that most equine rescue facilities rely on donations or personal funds to operate, it is imperative that horses are maintained economically and over-feeding is avoided. The UHC states that the cost to restore a horse to health in addition to maintain its normal needs ranges from $2,800 - $3,400 per year. The American Association of Equine Practitioners (AAEP) estimates the average annual cost of owning a healthy horse to be $2,500, not including fees associated with training, board, etc.

A promising solution to decreasing the occurrence of equine obesity and undernourishment, while also achieving maximum economic efficiency is the development of mathematical nutrition models that would allow horse owners to make more educated and precise feeding decisions. For beef and dairy cattle, nutritional models have been used in practice for decades to estimate the exact dietary energy intake needed to change BCS, allowing for more objective and accurate management of feeding programs. In the current horse industry, methods prescribed for affecting the BCS of a horse is vague due to the absence of a mathematical nutrition model. Recently, Cordero et al. (2013) developed such a model for horses. The model was structured similarly to those previously established for cattle, and results indicated that it was accurate in the prediction of BCS and BW changes. However, it was noted that more work is necessary to increase the accuracy of BF estimations (Cordero et al., 2013). Therefore, identifying an accurate, objective method of predicting BF in the current study will aid in the further development of such mathematical nutrition models.

Regarding the various components of body composition in horses, BF is certainly the most poorly predicted due to estimation procedures that are either subjective or dependent upon one anatomical location to create a prediction for entire BF content. The most widely used method for estimating live fat cover of horses is the Henneke BCS system which includes both visually and palpably appraising the horse at various anatomical locations and assigning it a numerical score (ranging from 1 to 9; Henneke et al. 1984). While this procedure has been useful in many facets including feeding programs and reproductive management, it is rather subjective, allowing for substantial evaluator error. Additionally, Dugdale et al. (2011a) found that BCS is unlikely to be a sensitive indicator for BF in moderately conditioned to obese equids. A more objective estimation of total BF is an equation using rump fat thickness determined via ultrasonography (Westervelt et al., 1976). While this method does prevent discrepancies accredited to evaluator subjectivity, it has been questioned as to whether predicting total BF composition based on one particular region allows for accurate estimations. More recently, deuterium oxide (D_2O) dilution has been validated as a means of accurately estimating BF in ponies (Dugdale et al., 2011b). However, further studies are warranted to confirm its efficacy in stock type horses because previous research in swine has found that D_2O dilution is only accurate in animals that share physiological resemblances (Rozeboom et al., 1994; Ferrel and Cornelius, 1984).

Although BCS serves as a useful tool to develop feeding programs and to optimize reproductive efficiency, the development of this method did not account for fatty acid (FA) composition of adipose tissues. Fatty acids are energy-dense molecules serving as an important energy source and integral component of cell membranes. Fatty

acids are stored in adipose tissues as triglycerides; therefore, they indicate the degree of obesity. Fatty acids also act as signaling molecules in regulating gene expression, some mechanisms of which remain unknown (Duplus et al., 2000). For example, n-3 FAs modulate transcription factors such as sterol-regulatory-element binding proteins and peroxisome proliferator-activated receptors. These factors are essential in controlling the expression of genes responsible for both systemic and tissue-specific lipid homeostasis (Deckelbaum et al., 2006). Such target genes also encode functional proteins in FA transport and metabolism, thereby ultimately affecting their propagation and composition profiles in tissues (Duplus et al., 2000).

Research indicates that the proportion of FAs in adipose tissues influences size of adipocytes with more n-3 and n-6 FA being associated with decreased cell size but greater proportion of dietary SFA being positively correlated with increased cell size and number (Garaulet et al., 2006). Enlargement of cells modifies their metabolic capacity, thus is involved in metabolic complications associated with obesity at whole body level (LeLay et al., 2001). Moreover, in dairy animals, milk FA composition is important for milk quality (Chilliard et al., 2007; Kelly et al., 1998) and have been reported to impact milk fat production (Chouinard et al., 1999) and health, growth (Hill et al., 2007), and body condition (Pedron et al., 1993) of off-spring.

Current literature lacks data of FA composition of horses. Research in humans suggests that n-3 FAs are important for health and disease prevention, including that of coronary heart disease and stroke, inflammation, and possibly behavioral disorders (Connor, 2000). King et al. (2008) suggested benefits from eicosapentaenoic and docosahexaenoic acids (Portier et al., 2006) could lessen the effects of exercise-induced

hypertension and pulmonary hemorrhage in horses. Furthermore, Vick et al. (2007) provided the first evidence establishing a relationship between obesity and inflammatory factors in horses. The authors suggested that an interrelationship between obesity, inflammatory cytokines, and insulin resistance existed and proposed additional research to describe the nature of these relationships. The etiology behind diseases such as insulin resistance and equine hyperlipemia remains unclear; therefore, research on FA composition of adipose tissues may yield further insight on such health issues. It was the rationale by the authors of the current study that FA composition of adipose tissues, especially visceral fat, would be important indicators of horse's health and growth and its correlation with equine BCS must be explored to provide foundation for the currently lacking, nonetheless essential, research.

Therefore, the primary objectives of the current study were to compare two BF prediction methods for stock-type horses by RFT and D_2O dilution with actual tissue fat analysis by near-infrared spectroscopy (NIR) and to identify the relationships among BF, BCS, and various physical measurements. The secondary objectives were to determine the FA composition of mesenteric (MS), cardiac (CD), subcutaneous (SC), intermuscular (IM), and leaf fat (LF) and to identify relationships between of FA composition and BCS in horses.

CHAPTER II

LITERATURE REVIEW

Body Condition Scoring

In recent years, researchers have learned more about the role BF plays in health issues such as insulin resistance and laminitis, in addition to reproductive efficiency. In order to more accurately conduct such research, a reliable method of determining total BF is crucial. Numerical scoring systems have been developed in cattle (Whitman, 1975) and horses (Henneke et al., 1983) to describe body condition and serve as a communication tool between producers, researchers, and extension personnel. The Henneke BCS system is a method of assessing fat cover in horses and assigning a numerical score (1-9) by visual and palpable appraisal of various anatomical locations. To develop this scoring system, 20 mature Quarter Horse mares of varying body condition were evaluated to determine areas of the body where fat cover appeared to accumulate. The locations selected included the crest of the neck, withers, area behind the shoulder, ribs, lumbar spinous processes, and tailhead (Henneke et al., 1983). This scoring system was modeled after the previously developed BCS system in cattle (Whitman, 1975) in which 1 represents extreme emaciation and 9 indicates obesity. To assess the accuracy of the BCS system as an indicator of total BF, Henneke et al. (1983) recorded BCS and other measurements including height, BW, and heart girth circumference for 32 mares of varying BCS at 90 days before foaling, 12 h post-parturition, and 90 days post-

parturition. Ultrasonic rump fat thickness (RFT) scans were taken according to methods prescribed by Westervelt et al. (1976) to estimate BF. Body condition scores were positively related ($r^2 = 0.65$, $P < 0.001$) to BF. No significant relationship between BW, height, or heart girth circumference and BF were observed, thus it was concluded that BCS was the most accurate physical measurement to use as a predictor of BF (Henneke et al., 1983). It is worth noting, however, the authors cited complications in applying the BCS system due to the presence of long, heavy winter hair coats and conformation differences (Henneke et al., 1983). Though the Henneke BCS system has proven to be very useful since its development, and currently remains the most widely used method of assessing body condition, it is based on visual appraisal, thus making it subjective in nature. Moreover, to determine BF in the aforementioned study, ultrasound predictions were used as opposed to actual whole carcass fat analysis, creating potential discrepancies in results pertaining to the relationship between BCS and BF. Therefore, there is a need to validate the use of BCS to predict BF in horses.

Wagner (1984) concluded that BCS was well correlated with BF when carcass fat was expressed on a total kg basis ($r = 0.91$; $P < 0.05$). Wright and Russel (1984) used 73 mature, non-pregnant, non-lactating cows of various genotypes to assess the value of BCS in providing a subjective estimation of BF, and thus its usefulness in increasing the precision of nutritional management in cattle. Results indicated significant relationships between BCS and chemically determined BF. It was noted, that differences between genotypes in fat deposition patterns accounted for differences in the relationship between BCS and BF. For example, British Friesian cows carried more fat in their intra-abdominal depots and had a lower proportion of subcutaneous fat, thus causing them to be fatter at

any given BCS. Conversely, Hereford × Friesian cows carried the most subcutaneous fat resulting in their being the least fat at any BCS (Wright and Russel, 1984).

In sheep, similar BCS systems have been in place since the 1960's. Russel et al. (1969) developed a systematic method of assessing body condition in sheep which entails palpating the muscle and fat along the lumbar vertebrae of the spine, in particular the spinal processes and the loin eye muscle area, and assigning a score from 1-5. This system is particularly useful when monitoring pregnant and lactating ewes, as well as growing lambs. Subjectively assessed BCS determined on the live animal were related to BF in 30 adult Scottish Blackface ewes, thus validating the usefulness of BCS in estimating proportions of fat in live sheep and its superiority in doing such when compared to using BW alone (Russel et al., 1969). Phythian et al. (2012) analyzed the reliability of body condition scoring of sheep for cross-farm assessments. While the majority of sample sheep used in this study fell within the mid-range of body condition (BCS 2 - 3), which possibly affected the analysis of the collected data, it was concluded by the authors that trained and experienced assessors using both half- and whole-unit increments were able to reliably score BCS of sheep. It was also noted that a re-calibration exercise may improve the consistency of cross-farm assessments between different accessors (Phythian et al., 2012).

Nutritional Management

In addition to the subjectivity of the BCS system, it is relatively difficult to accurately determine the changes in dietary energy intake needed to increase or decrease body condition based on visual appraisal alone. For beef and dairy cattle, mathematical models have been developed to effectively estimate energy requirements needed to

maintain an animal at a specific body condition. Furthermore, these models have prompted the growth of decision support systems for the purpose of helping cattle producers maintain a herd requiring the lowest economic expenditures possible (Tedeschi et al., 2004). Recently, a study was conducted to develop a similar mathematical nutrition model for horses (Cordero et al., 2013). Using data collected from 24 Quarter Horse mares (Cavinder et al., 2009), researchers created a model for horses which would estimate exact DE requirements needed to alter BCS in horses. To test the precision of the model, 20 non-lactating Quarter Horse mares were divided into 4 treatment groups dependent upon pretrial BCS. Each group was fed to alter BCS by 1 unit as follows: Group 1 went from BCS 4 to 5, Group 2 went from BCS 5 to 4, Group 3 went from BCS 6 to 7, and Group 4 went from BCS 7 to 6. Body condition score, RFT, and BW were recorded for each mare before the feeding trial started, and once a week after that for the duration of the 30 d feeding trial. Initial and intended BCS, BF, and BW data were recorded for each mare and inputted into the model. Mares were then fed according to the energy suggestions put forth by the model to reach the targeted BCS in a 30 d time period. It was concluded that the developed model was precise in the prediction of BW (r^2 = 0.94) and BCS (r^2 = 0.907) changes; however, improvement is needed for the measurement of initial and prediction of final BF content (Cordero et al., 2013). The reliability of this model in horses depends on the precision of body content estimations and a positive outcome to the current project will validate a precise method of predicting BF, thus benefitting the further development of such a mathematical nutrition model.

Body Condition and Reproductive Function

For decades, there has been an observed relationship between body condition and reproduction in livestock. Early observational research in horses indicated that in order to maximize reproductive efficiency, mares should be in moderate to high body condition at the time of breeding (Day, 1939). In addition to developing the BCS system, Henneke et al. (1984) documented a positive relationship between reproductive efficiency and body condition in horses. Specifically, these authors found that mares entering the breeding season at a BCS of 5.0 or higher exhibited shorter intervals from parturition to ovulation, higher rates of pregnancy until 30 d following ovulation, and increased rates of maintained pregnancies to 90 d post-breeding. Performance was similar between horses in a moderate and fat body condition, while mares in a thin BCS (< 5) experienced a delay in onset of estrus and ovulation (Henneke et al., 1984). Another consequence of low body condition in mares around foaling is a longer average gestation period when compared to mares in a BCS of ≥ 6 (Hines et al., 1987). Furthermore, Hines et al. (1987) found that the interval from parturition to ovulation in thin mares (BCS ≤ 4.5) was random whereas mares in a BCS of ≥ 6 had predictable intervals from parturition to ovulation. Gentry et al. (2002) assessed the interaction of BCS with equine somatotropin, GnRH analog, and dexamethasone, finding a low BCS had significant implications on the hormonal status and reproductive traits of mares during the seasonal anovulatory period. Mares maintained in a low BCS (3.0 - 3.5) exhibited no ovarian stimulation from treatment of somatotropin (eST) after the administration of GnRH analog. Mares in a low BCS also showed virtually undetectable levels of leptin, a hormone secreted by the adipocyte and thought to help control satiety. Mares with a moderate to high BCS (avg =

7) displayed increased reproductive function in addition to heightened leptin levels after treatment with dexamethasone (Gentry et al., 2002). A study of 119 Lusitano mares, ranging from 4 to 22 yr of age, investigated the effects of changes in body condition on reproductive performance in different breeding systems. It was found that body condition of mares at conception had a significant effect on fertility at the first 2 postpartum estrous cycles. Fertility was optimized at a BCS of 3.0 on a 1-5 scale (Carroll and Huntington, 1988). Additionally, foals that were nursed by mares with negative changes in body condition during the first 3 mo of lactation exhibited lower growth performances than foals nursing off dams in a maintained or increased BCS (Fradinho et al., 2014). It should be noted here that the latter data of the aforementioned study does contradict with previous findings which observed a similarity in foal weights after 90 d of lactation in foals suckling mares in decreasing body condition versus those in a maintained or increasing body condition (Henneke et al., 1984). Although ideal body conditions likely vary somewhat among individual animals, there is evidence that maintaining a horse in a high BCS yields no advantage in reproductive traits over mares in a moderate BCS (Cavinder et al., 2007). Researchers compared the postpartum endocrine profiles of 24 Quarter Horse mares in fat versus moderate body conditions. Mares were equally divided into 2 groups: moderately conditioned (BCS of 5 - 6) and fat (BCS of 7 - 8). Blood samples were collected from mares daily from the time of foaling up to the second postpartum ovulation. There was not a significant difference in serum leptin between the 2 groups at any point during the time between foaling and the second postpartum ovulation. Serum T_4 levels did differ, with moderately condition mares exhibiting higher concentrations than fat mares. Lastly, IGF-1 concentrations varied greatly between the 2

groups throughout the postpartum estrous cycle. Close to the first postpartum ovulation, fat mares had significantly higher levels of IGF-1, remaining elevated through the interovulatory period and in the time approaching the second postpartum ovulation. It was concluded that the moderately conditioned mares possess adequate leptin concentrations to reproduce, since leptin is thought to be a signal to the animal whether it has an appropriate energy reserve to accommodate reproduction. The variance in T_4 levels and IGF-1 concentrations suggest that, in a mare at a BCS of 5, the elevation of T_4 along with the decline of IGF-1 may be markers of possible reproductive inefficiency (Cavinder et al., 2007). This data may be useful in more precisely determining the ideal BCS necessary to optimize reproductive performance, while avoiding unnecessary over-feeding.

Leptin

Leptin is an adipocyte hormone discovered in 1994 and is thought to serve a wide variety of biological functions. A major metabolic function of leptin is helping maintain homeostasis by providing the hypothalamus information pertaining to BF, thereby altering central nervous system (CNS) functions known to regulate feed intake and energy balance (Prolo et al., 1998). Given the pertinence of leptin's metabolic functions, it has been hypothesized that this hormone may be advantageous in objectively assessing body condition in the horse. Buff et al. (2002) were among the first to examine leptin's impact on BCS in horses. After successfully cloning partial sequences of equine leptin and leptin receptor genes and quantifying peripheral concentrations of leptin in equine tissue, they aimed to determine if peripheral concentrations of leptin correlate with BCS in horses and to assess whether changing BCS would influence leptin concentrations in

horses. Results indicated that serum concentrations of leptin increased as BCS increased ($r = 0.64$, $P = 0.0001$) and concentrations of leptin were greater in geldings and stallions than mares ($P = 0.0002$). In the same study, 18 pony mares were assigned to lose or gain weight over a 14-wk time period. Blood samples, BW, and BCS were collected from each mare every 14 d. While there were statistically significant changes in BCS, corresponding statistical changes in peripheral leptin concentrations were not seen. Authors noted that this was most likely due to the small range of BCS alteration. The data did signify a tendency for leptin concentrations to be greater in fat, restricted mares than in thin, supplemented mares ($P = 0.09$). Therefore, it was concluded that fat mass in horses significantly influences peripheral leptin concentrations (Buff et al., 2002). In another study, researchers tested the hypothesis that adipocytokines (signaling proteins secreted by the adipose tissue) are related to adiposity in horses (Kearns et al., 2006). Data collected from 23 mature mares and 12 weanling fillies suggested that leptin is proportional and adiponectin (protein functional in regulating glucose levels and fatty acid breakdown) is inversely proportional to fatness in horses (Kearns et al., 2006). Based on the limited amount of research available pertaining to the relationship between adiposity and leptin concentrations in horses, it seems possible that leptin may be beneficial in the prediction BF. Nevertheless, this specific area of study is certainly in need of further exploration.

Health Issues Associated with Obesity & Implications of Undernourishment

In addition to the relationship between body condition and reproduction, fat content has also been linked to metabolic issues. Conditions such as insulin resistance and laminitis in particular are often associated with obesity. Although this relationship is

well established, the exact etiology behind these issues in horses remains vague. Research has proven that adipose tissue is not merely a storage organ for excess calories, but it is living metabolic tissue that has inflammatory effects and is active in secreting hormones that are functional in energy balance (Vick et al., 2007). An initial study conducted by Vick et al. (2007) observed that obese mares exhibited higher levels of circulating insulin and lower insulin sensitivity (insulin resistance). In addition, the study was the first to associate obesity with increased inflammatory cytokines in the horse as well as an interconnection between obesity, inflammatory cytokines, and insulin resistance. Insulin resistance has been strongly associated to debilitating laminitis (Coffman and Colles, 1983; Pass et al., 1998; Geor and Harris, 2009) and irregular reproductive function (Vick et al., 2006). It has also been suggested that obesity may actually be a low-grade systemic inflammatory disease (Das, 2001; Ramos et al., 2003). The aforementioned findings have contributed to the further understanding of BF, which is poorly defined, thus narrowing the gap of knowledge regarding the exact etiology behind obesity-related metabolic issues.

Undernourishment in horses leads to a variety of consequences, depending on severity. Horses in good health and appropriate body condition may survive up to 60 d with complete absence of food, however, once a starved animal has lain down for more than 72 h, the chance of mortality is high (Argo, 2013). Research in 10 Shetland ponies has also indicated that restricting feed over an extended period of time not only negatively affects body mass and BCS, but also leads to significant changes in some blood parameter concentrations that are known to be associated with the health of an animal (Brinkmann et al., 2013). In this study, the 10 ponies were separated into 2 equal

groups of 5. Over a period of 7 mo, all animals were monitored for blood concentrations of non-esterified fatty acids (NEFA), total protein (TP), total bilirubin (TB), beta-hydroxybutyrate (BHB), and T_4. Additionally, changes in BCS and body mass were observed. Five of the 10 ponies were fed restrictively for 4 out of the 7 mo to imitate potential feed shortages during winter months for horses kept in semi-natural housing systems. The ponies on restricted feed lost, on average, 18.4% of their body mass, and BCS decreased by an average of 2.2 units (1-5 scale). Blood concentrations of TB in restrictively fed ponies continuously increased, as did NEFA concentrations (indicating fat was mobilized), while TP and BHB levels decreased in the feed restricted group only towards the end of the trial. It was concluded that BCS, plasma NEFA levels, and TB concentrations may help rapidly detect health issues caused by undernourishment such as insufficient energy reserves (Brinkmann et al., 2013; Powell et al., 2000). Additionally, horses that lack consistent meals may suffer from adverse health effects indirectly caused by deficient nutrition through behavioral abnormalities. For example, horses that are confined and do not receive ample amounts of forage may develop cribbing behavior, which leads to decreased availability of saliva in the stomach, potentially causing ulceration (McCall et al., 2009). Inadequate feed intake eventually causes depleted glucose availability resulting in an increased occurrence of fat and protein breakdown into fatty acids and amino acids, respectively, to meet energy needs (Brinkmann et al., 2013). Evident by the previously mentioned study, plasma NEFA concentrations increase as a result of lipid store utilization for energy (Brinkmann et al., 2013; Heitmann et al., 1986). A small portion of NEFA is normally utilized for energy by tissue (Brinkmann et al., 2013; Blum et al., 1983); however, the majority is filtered through the liver where it is

converted to ketone bodies (acetoacetate and BHB) which serve as essential metabolic fuel for tissues. Under normal circumstances, the brain would use glucose as its primary energy source, however, during starvation, ketone bodies replace glucose for that purpose. A high ketone concentration may lead to starvation acidosis. Nevertheless, in horses, ketogenesis is limited to facilitate free fatty acids being re-esterified into triglycerides. Thus, high triglyceride concentrations may cause hyperlipidemia, or an excess of lipids in the blood (Brinkmann et al., 2013; Sjaastad et al., 2003).

Deuterium Oxide Dilution

Thus far, evidence of a strong association between BCS and actual BF content in horses has been sparse, due to the consequential and laborious process of carcass dissection that is necessary to quantify BF definitively. Additionally, methods of quantifying BF are outdated and speculated to be somewhat inaccurate. Recently, research using 7 mature Welsh Mountain pony mares indicated that deuterium oxide (D_2O) dilution is a valid means of objectively estimating total BF mass in equids (Dugdale et al., 2011b). In the aforementioned study, researchers compared D_2O dilution-derived estimates of total body water (TBW) and BF to values obtained from proximate analysis and carcass dissection. They hypothesized that D_2O dilution would offer a minimally-invasive and accurate means of measuring TBW and BF in horses. Their results indicated that TBW and BF values attained by D_2O dilution were strongly associated with proximate analysis and dissection derived values. This study was the first to verify the accuracy of D_2O dilution for the objective and repeatable measurement of total BF in living ponies (Dugdale et al., 2011b).

Factors that must be considered when utilizing D_2O dilution include minimalizing the equilibration time for the hydrogen isotope, the use of a precise and effective assay for the isotope, and curtailing water loss during fabrication and dissection of the carcass (Rudolph et al., 1988). Research in neonatal pigs that followed the techniques prescribed above validated that D_2O dilution accurately reflects TBW, and therefore can be used to estimate body protein and fat content (Rudolph et al., 1988). The efficacy of D_2O dilution has also been examined in growing swine (Shields et al, 1983). Results from the D_2O analyses of 73 pigs were compared with total and empty (excluding gut) body water values taken from chemical analysis of the ground animals. It was validated that D_2O can be used for the in vivo estimation of empty body content following a method that involves estimating empty BW from live weight and predicting empty body water from D_2O space (Shields et al., 1983). Principally, the inverse relationship of body fat to water, in addition to total BW, was used to precisely quantify BF. A prediction equation for empty BF in pigs derived from empty body water and BW was proposed by Shields et al. (1983):

$$\% \text{ fat} = -.988\ (\% H_2O) - .124\ (\text{wt.}) + .001\ (\text{live wt.})^2 + 81.992 \qquad 2.1$$

Furthermore, D_2O dilution has been shown to be useful in estimating BF content in reproducing swine (Shield et al., 1984). Seventy-two crossbred gilts were separated into 2 groups. Forty gilts were hand bred, and subsequently divided into 4 subgroups that were infused with D_2O at either 57 or 105 days after being bred and 5 or 25 days postpartum. The remaining 32 gilts were not bred and infused with D_2O at the onset of estrus and at times correlating with the pregnant animals (Shields et al., 1984). Results from this study, and studies with growing swine (Shields et al., 1983) and neonatal pigs (Rudolph et al.,

1988) suggest that D_2O dilution method may be a useful in predicting BF content of pigs in all physiological stages.

While the previously mentioned study by Dugdale et al. (2011b) validated the use of D_2O dilution to predict TBW and BF in ponies, further studies are warranted to confirm the usefulness of this method in stock type horses. Research in 58 mature gilts has suggested that prediction equations involving D_2O dilution are solely accurate in animals that are physiologically similar to the animals in which the equation was derived. Before slaughter, pigs were weighed, scanned for 10^{th} rib backfat thickness via ultrasound, and administered D_2O. Deuterium oxide space was calculated from body water D_2O concentration established at equilibrium, reached at 150 and 210 min following administration. Regression models predicting empty body composition components were fixed involving all possible combinations of variables (D_2O space, live weight, and/or backfat thickness). The study concluded that age, physiological and reproductive status, genotype, and nutritional history should be taken into account when using a single predictive equation to estimate body composition in a population of animals (Rozeboom et al., 1994). Research performed on 60 pigs of diverse types reinforces the need to take genotype, nutritional history, and physiological status into consideration when applying a BF prediction equation using D_2O dilution to a population of animals. Equations were developed that related weight of body components (i.e. water, fat) to D_2O space and live weight. However, these equations were shown to be significantly influenced by pig type. It was concluded from this study that, due to the observed inconsistency in the relationship between D_2O space and body water, using D_2O

dilution to predict body water is unlikely to yield accurate estimations of body composition in animals of differing genotypes (Ferrel and Cornelius, 1984).

In contrast to swine, the use of D_2O dilution in cattle has proved less accurate in predicting body composition. The basic concept behind D_2O dilution includes the injection of a known quantity of a biological tracer (D_2O) into the blood and the estimation of TBW based on the equilibration of the D_2O and body water. In cattle, this equilibration is complexed by the large amount of water held in the gastrointestinal tract in comparison to that of non-ruminant animals. Additionally, the water content of gastrointestinal tracts varies substantially between ruminants based on dietary intake, environmental factors, and physiological differences among animals. The issue lies in the fact that D_2O passes into the gut water just as it does tissue water, creating a substantial error in the estimation of empty body composition, when TBW is assumed to be equal to empty body water (Arnold et al., 1985). A one-compartment model involving D_2O dilution was effective in generating repeated measurements of body composition in beef steers, however, limitations to this model exist due to differences in gastrointestinal tract contents (Arnold et al., 1985). Nevertheless, in a study conducted with feedlot cattle to determine the effects of various nutritional and physiological factors on body composition, D_2O dilution was used to assess compositional differences (McCarthy et al., 1985).

In sheep, similar ruminant-related complications involving D_2O dilution exist. Due to the large quantity of water held in the gastrointestinal tract, estimation of TBW and, therefore, body fat content may be compromised. Alike issues also arise in pregnant animals as a result of the large proportion of body water held in the uterus (Flanagan,

1964). Research in 14 Blackface ewes did show D_2O dilution to be equally accurate in body composition estimation in pregnant ewes and non-pregnant ewes; however, the precise measurement of TBW was crucial to results (Foot and Greenhalgh, 1970). A study conducted with fat-tailed Barbary ewes indicated that D_2O dilution was similar in BF prediction accuracy to thin-tailed ewes, and that the characteristic fat containing tail did not significantly affect the relationship between TBW and BF (Atti et al., 2000). In the same study, researchers found that the body composition prediction equation that was developed involving D_2O dilution was more accurate than the equation using BCS and/or BW (Atti et al., 2000).

Due to the varying success of D_2O dilution in other livestock species, as well as the various physiological factors that have shown to be influential to its effectiveness, further validation of this BF estimation method in stock type horses is certainly warranted.

Ultrasound Techniques

Body composition estimation via ultrasonic measurements has been the focal point of a considerable amount of research in various species. In horses, Westervelt et al. (1976) suggested that measuring RFT using ultrasound technology is a useful method of estimating total BF. Researchers conducted a series of 4 experiments to determine the relationship between ultrasonic measurements of fat to actual fat cover, the usefulness of said measurements in predicting total BF, and the influence that exercise and dietary intake have on fat cover in horses and ponies. In the first experiment, 8 ponies were fed ad libitum and 7 ponies were fed the same diet at a restricted amount for 4 ½ mo. In these animals, ultrasound measurements of rump fat thickness were highly correlated with

actual RFT. In the third experiment, 8 horses were measured by ultrasound for RFT and then slaughtered and assessed for chemically extractable fat. Rump fat thickness was highly correlated with chemically extractable fat and an equation was developed to describe the relationship: Y = 8.64 + 4.70 X (r^2 = 0.86) where X represents the ultrasonically determined rump fat thickness (cm) and Y corresponds to extractable fat percentage (Westervelt et al., 1976). Although ultrasonic measurements have subsequently been used in several studies to determine BF (Hines et al., 1987; Cavinder et al., 2007; Cordero et al., 2013; Silva et al., 2005), the reliability of this method has been questioned as fat depots have been known to vary among individual horses. It is worth noting Westervelt et al. (1976) used a relatively small trial population of only 8 horses to determine the relationship between ultrasonic measurements and total BF.

Physical Measurements

For years, one of the most widely accepted methods of assessing BW in equids in the absence of a scale is the use of weight tapes and BW estimation formulas. According to Wagner and Tyler (2011), the most widely used formula for such estimations is: weight (kg) = (heartgirth cm^2 × body length cm)/(11,880 cm^3). Nevertheless, several modifications of this formula have been used, mostly varying in anatomical measurement points. Less attention, however, has been devoted to estimations of BF based on anatomical measurements such as girth circumference, body length, neck circumference, and height. In a recent study by Carter et al. (2009), researchers examined the possible relationships between apparent adiposity, BCS, and morphometric measurements in horses and ponies. They recorded BW, height, length, girth and abdominal circumference, neck length, neck crest height, neck circumference, cresty neck score

(CNS) and BCS of 34 horses and 75 ponies of various breeds. Additionally, blood samples were analyzed for insulin, glucose, leptin, and triglycerides. Results indicated that CNS had possible physiological implications as it was strongly associated with insulin concentrations. Moreover, they concluded that girth:height ratio was the most accurate in prediction of overall adiposity as it was most strongly associated with BCS and blood parameters such as leptin (Carter et al., 2009). While the aforementioned study found various body measurements to be associated with subjective BCS, the authors did not examine the relationship between such measurements and BF evaluation via carcass dissection. Recently, Dugdale et al. (2011a) conducted an experiment to define the relationship between BCS, morphometric measurement indices of BF, and measurements of actual body carcass composition. They predicted that BCS and morphometric measurements provide an accurate method of non-invasively measuring BF in horses. Results indicated that heart girth:withers height (body girth) and ultrasonically determined retroperitoneal fat depth were closely associated with total chemically extracted lipid. It was concluded that BCS, alone, is likely not an accurate means of assessing BF, however morphometric measurements of body girth and retroperitonel fat depth may be useful in augmenting subjective BCS systems (Dugdale et al., 2011a).

Fatty Acid Composition

Fatty acid composition of muscle and adipose tissue in food animals has been extensively studied largely due to the nutritional implications for consumers. While excessive amounts of fat in meat is typically considered unfavorable due to health concerns, the fatty acid composition and total fat content of both adipose and muscle tissue are important for nutritional value and quality of meats. Several studies indicated

that sources of dietary lipid have a significant impact on tissue fatty acid profiles in various livestock species (Scollan et al., 2001; French et al., 2000; Nuernberg et al., 2005; S. John et al., 1987; Otten et al., 1993). Caldeira et al. (2007) recorded higher serum concentrations of NEFA in ewes in a state of undernutrition (BCS = 1.25 or 2 on a 1 – 5 scale). Furthermore, they concluded that of the variables tested as metabolic indicators, NEFA (along with glucose and insulin) concentrations proved to be most reliable in diagnosing the ewe's energy status (Caldeira et al., 2007). Data collected from pigs, sheep, and cattle by Enser et al. (1996) indicates that adipose tissue has considerably higher fatty acid content than muscle; however, the fatty acid composition of both tissues is generally similar. It was noted that there are important differences among species. According to these authors, pigs exhibited higher concentrations of the major PUFA (polyunsaturated fatty acid), linoleic acid, than cattle and sheep. Although these authors reported similar proportions of linoleic acid in both adipose and muscle tissue, greater proportions of this FA in pig adipose tissue than in muscle tissues has been reported by others (Teye et al., 2006a; Teye et al., 2006b). Conversely, in sheep and cattle, linoleic acid is present in higher levels in muscle tissue than adipose (Wood et al., 2008). Derived completely from the diet, linoleic acid in pigs is not altered in the gastrointestinal tract, but is absorbed through the small intestine into the blood stream where it is then incorporated into the tissues. In ruminants, this PUFA is consumed in high levels through concentrate feedstuffs and is broken down into MUFA and SFA in the rumen. This leaves only a small percentage (about 10%) of linoleic acid for uptake by the tissues (Wood et al., 2008). Typically, the meat of ruminant animals is higher in SFA in comparison to monogastric animals due to rumen microbial biohydrogenation (De Smet et al., 2004). In

beef cattle, the second most important PUFA, α-linolenic acid, is a major fatty acid consumed in the diet as it comprises over 50% of all fatty acids in grass and grass commodities (Wood et al., 2008).

As fat content of the animal increases, the fatty acid profile of the animal is altered. Wood (1984) demonstrated this in pigs, where stearic and oleic acid increased and linoleic acid declined in subcutaneous adipose tissue as the animal deposited more fat approaching slaughter. The authors attributed this to an increasing occurrence of de novo tissue synthesis of MUFA and SFA in addition to less direct incorporation of dietary linoleic acid (Wood, 1984). Another study found similar results where stearic and oleic acid increased and linoleic decreased as pigs were fed a control diet for 20, 60, or 100 d (Kouba et al., 2003). Several studies in pigs have documented an inverse relationship between proportions of linoleic acid in subcutaneous adipose tissue and the amount of total body fat (Wood et al., 1978; Wood et al., 1989).

Many of the differences in fatty acid composition that are observed between breeds and genotypes are due to differing fatness levels (De Smet et al., 2004). Changes in fatty acid composition between animals of different fatness levels are different between pigs and cattle. Generally, SFA and MUFA content increases faster as fatness increases in comparison to PUFA, resulting in a decrease in the PUFA/SFA (P/S) ratio (De Smet et al., 2004). In beef cattle, decreasing total body fatness is more efficient in increasing the P/S ratio than dietary alterations as a sharply increasing P/S ratio has been observed at low levels of intramuscular fat (De Smet et al., 2004). Similarly, intramuscular fat level also affects the P/S ratio in pigs, however, unlike in beef cattle, dietary changes weigh more heavily (De Smet et al., 2004).

Various tissues do vary in their fatty acid composition. Typically, saturation levels are greater in tissues deeper in the body compared to more superficial depots such as subcutaneous adipose tissue (De Smet et al., 2004). Researchers have also observed fat depot specific differences in fatty acid profiles of humans (Garaulet et al., 2006; Malcolm et al., 1989). In a study of > 700 autopsied human adults of varying races and ages, fatty acid compositions of 3 fat depots including one deep-seated site (perirenal), and two subcutaneous sites (abdominal and buttock) were examined. Results indicated that saturated fatty acids were greatest in perirenal adipose tissue and least in subcutaneous buttock adipose tissue. Conversely, MUFA were least in perirenal adipose tissue and greatest in subcutaneous buttock adipose tissue. Abdominal subcutaneous adipose tissue was intermediate in proportions of SFA and MUFA between the aforementioned sites (Malcolm et al., 1989).

CHAPTER III

BODY FAT OF STOCK-TYPE HORSES PREDICTED BY RUMP FAT THICKNESS AND DEUTERIUM OXIDE DILUTION AND VALIDATED BY NEAR-INFRARED SPECTROSCOPY OF DISSECTED TISSUES.

Introduction

Body condition score of horses is positively correlated with reproductive efficiency (Henneke et al., 1984; Kubiak et al., 1987) and is indicative of metabolic issues (Vick et al., 2007). However, body fat (BF, %) in horses may be poorly defined and predicted because current procedures are either subjective (Henneke et al., 1983) or dependent upon one anatomical location (Westervelt et al., 1976). The most commonly used method to assess the degree of fatness in horses is the BCS system, which employs both visual and palpable appraisals of various anatomical locations using a numerical score ranging from 1 to 9 (Henneke et al., 1983). Although this procedure is useful in the assessment of overall energy status, its subjectivity can result in variation among evaluators. Additionally, data defining the relationship between BCS and BF is sparse. An objective BF prediction method using RFT determined by ultrasonography was proposed by Westervelt et al. (1976). Despite the method's objectivity, the accuracy of it has been questioned because a single location is unlikely an accurate representation of fat deposition patterns of horses differing in breed and production stage (growth, reproduction, work, etc.). According to the BF prediction equation proposed by these

authors (BF = 8.64 + 4.70 × RFT), a horse with no RFT has 8.64% BF, a markedly high value compared to data from the current study. Recently, deuterium oxide (D_2O) dilution has been validated as an accurate estimation of BF in ponies (Dugdale et al., 2011b). Further research is warranted to confirm its accuracy in stock-type horses as previous studies in swine show D_2O dilution predictions to be accurate only in animals with shared physiological resemblances (Rozeboom et al., 1994; Ferrel and Cornelius, 1984). Therefore, the objectives of the current study were to:

1. Compare two methods of predicting BF by RFT and D_2O dilution with actual tissue fat analysis by near-infrared spectroscopy (NIR) in stock type horses, and
2. To identify the relationships among BF, BCS, and various physical measurements.

Materials and Methods

The current study was conducted under an approved Mississippi State University Institutional Animal Care and Use Committee protocol #15093.

Experimental Design

Twenty-four stock-types horses were selected based on 3 primary criteria: geriatric, crippled, and/or unsafe. In the current study, "geriatric" was defined as 15 yr of age or more, "crippled" was defined as being chronically unsound, and "unsafe" was defined as being a significant risk to the wellbeing of human handlers. Horses were evaluated and separated into groups according to BCS. Horses were maintained on native grass pasture for 4 mo leading up to time of euthanasia. Fourteen horses began the current study at desired BCS of 4, 5 or 6, thus continued to be maintained on pasture until

slaughter. The remaining 10 horses were fed with concentrate (Nutrena Strategy, Nutrena Animal Nutrition; Minneapolis, MN) twice per d for 30 d (Cordero et al., 2013) in addition to being maintained on the similar pasture until desired BCS was reached. Two horses (BCS 1 and 2) failed to meet intended BCS of 3. Approximately 20 h before slaughter, horses were weighed and confirmed to be of BCS 1 (n = 1; 433 kg), 2 (n = 1; 415 kg), 3 (n = 1; 376 kg), 4 (n = 7; 468 ± 13 kg), 5 (n = 10; 455 ± 11 kg), and 6 (n = 4; 493 ± 12 kg; Henneke et al., 1983) and RFT was measured via ultrasonography (Aloka Co., LTD, SSD500V; Tokyo, Japan) over the rump, at the center of the pelvic bone, 10 cm from point of hip, and 5 cm lateral from the midline (Westervelt et al., 1976). Body height (HT), length (BL), neck circumference (NC), and girth circumference (GC) were also recorded at this time.

Analysis of Deuterium Oxide

Immediately following the documentation of all physical measurements, a jugular catheter was placed intravenously to facilitate D_2O infusion. Deuterium oxide dose was 0.12 g/kg of BW (Dugdale et al., 2011b). Deuterium oxide was administered via the jugular catheter over a time period of 15 to 60 s, followed by an infusion of 10 ml of sterile saline to ensure D_2O dispensation. Blood was collected immediately before and 4 h after D_2O administration via venipuncture of the ipsilateral jugular vein into a vacutainer tube (BD Vacutainer®, Franklin Lakes, NJ). Food and water were withheld during the 4 h period before and after D_2O administration to allow D_2O equilibration. Plasma was immediately collected by centrifugation (15 min, 2500×g, and 5°C) and stored in 4.5 mL microcentrifuge tubes at -80°C for subsequent D_2O analysis. Thawed plasma samples were analyzed by gas isotope ratio mass spectrometry according to

methods of Dugdale et al. (2011b), after plasma was filtered, and water in the filtrate containing hydrogen isotopes underwent zinc reduction. Deuterium abundance was converted to parts per million so that body water content (%) and BF could be calculated. A body lean hydration factor of 0.732 suggested by Pace and Rathbun (1945) was used (Dugdale et al., 2011b).

Animal Slaughter

Before slaughter, horses were held in a dry lot for approximately 12 h with *ad libitum* access to water. On the day of slaughter, horses were individually sedated (1.1 mg xylazine/kg BW) and anesthetized (2.2 mg ketamine/kg BW) and KCl solution was administered to cease cardiac function. Horses were then exsanguinated by severing the carotid and jugular veins and allowed to bleed for at least 20 min. Carcasses were then transported to the Mississippi State University College of Veterinary Medicine Necropsy Laboratory for further processing. Carcasses were hoisted to record dead weight (DW, kg), then lowered to remove the head at atlanto-occipital joint, tail between the last sacral and the first caudal vertebrae, and forefeet at the knees with shanks being left with carcasses. Carcasses were hoisted again for evisceration and hide removal, and HCW was recorded. Subcutaneous fat attached to the hide was collected. Hot carcasses were split into 2 sides by sawing vertically through the vertebrae, then lowered and sawed at the hocks to remove the hind feet. The right side was then cut into neck and shoulder, thoracic, lumbar, rump, shank, plate, chest, and flank sections, double-bagged, and stored at -20°C until subsequent dissection and analysis. Fat from organs (e.g., heart) and intestines (mesenteric) were collected and total weight as visceral fat was recorded. Abdominal fat was collected and weight was recorded as leaf fat. All fat tissues were

combined as a separable fat section, which was treated similarly to other anatomical sections during NIR analysis. Weights of the tail, lungs, esophagus and trachea, pancreas, adrenal glands, stomach, empty stomach, small intestine, empty small intestine, large intestine, empty large intestine, cecum, empty cecum, spleen, hide, head, forefeet, hind feet, kidney, heart, liver, diaphragm, bladder, reproductive tract, and mammary gland were also recorded.

Analysis of Fat in Soft Tissues

Carcass sections were thawed in refrigerators at 4°C and purge was calculated by weight difference between wet and dry bags, however, was too minute to be included in calculation because of high pH of tissues frozen pre-rigor. Soft tissues were manually separated from bone. Bone and separable tissues were weighed separately for each section. Separable tissues were ground twice (ProCut KG-22W-XP Meat Grinder, Davison's Butcher Supply, Commerce, CA), through 0.95 cm (3/8-in) and 0.32 cm (1/8-in) extruder plates, consecutively. Three random samples of approximately 0.45 kg (1 lb) of ground tissues were collected, vacuum-packaged, and stored at -20°C for subsequent NIR analysis of fat, moisture, collagen, and protein contents (%). Each sample was analyzed in triplicate (FoodScan™ Pro/Lab, Type 7880; Foss, Eden Prairie, MN). Fat content of 15 randomly selected samples with a wide range of fat content was also determined by chloroform-methanol extraction (Luna et al., 2011) to validate the accuracy of NIR data.

Body Fat Calculation

Body fat determined by RFT (RF, %) was calculated by using the prediction equation developed by Westervelt et al. (1976), as follows:

$$RF\ (\%) = 8.64 + 4.70 \times RFT\ (cm) \quad 3.1$$

Deuterium oxide abundance was used to calculate BF as described by Dugdale et al. (2011b). Briefly, D_2O abundance was converted to a parts-per-million value, which was used to determine body water mass (kg) and body water content (%) with a 4% correction factor for isotopic exchange between D_2O and other non-water components. Total water was used to calculate percentage of fat-free body by using a lean tissue hydration factor of 0.732. Body fat predicted by D_2O dilution (D_2OF, %) was calculated as follows:

$$D_2OF\ (\%) = 100 - \text{percentage of fat-free body} \quad 3.2$$

Body fat by dissection and NIR analysis was calculated by dividing weight of total tissue fat (TF, kg) by appropriate animal weights, as defined below, and multiplying by 100. Tissue fat was calculated as follows:

$$TF\ (kg) = \Sigma(f_i/100) \times m_i \quad 3.3$$

with f_i being fat content (%) of each anatomical section by NIR analysis and m_i being weight (kg) of each section. Fat from head, feet (below knees and hocks), hide, tail, visceral tissues (except for separable fat previously mentioned), and bone were considered to be minute compared with fat from soft tissues and separable internal fat (abdominal, pelvic, and organ fats), and therefore, were not processed for fat analysis. The animal weight bases for BF were LW, DW, LW with empty gut (LWEG, kg), and DW with empty gut (DWEG, kg). Except for LW and DW being recorded, LWEG and DWEG were calculated by subtracting gut content from either LW or DW. Gut content

was calculated from weights of full and empty gut sections (Table 4). Body fat based on animal weight, i.e. LW (LWF, %), DW (DWF, %), LWEG (LWEGF, %), or DWEG (DWEGF, %), was calculated as follows:

$$\text{LWF, DWF, LWEGF, or DWEGF (\%)} = 100 \times (\text{TF/animal weight}) \quad 3.4$$

Statistical Analysis

Eighteen horses (n = 4 for BCS 4, n = 9 for BCS 5, and n = 5 for BCS 6) were used for statistical analysis, excluding BCS of 1 (n = 1), 2 (n = 1), and 3 (n = 1), as well as 3 horses of BCS 4 with abnormal BF (-3.14, 1.31, and 1.36%) based on D_2O and NIR analyses. Principal component analysis was used to reduce BF variables, including D_2OF, RFT, RF, TF, LWF, DWF, LWEGF, and DWEGF to 2 principal components (PCs) while preserving total variance in the data. The loadings, i.e., correlation coefficients of BF variables with PC1 (horizontal coordinate) and PC2 (vertical coordinate) were used to map these variables in a bi-plot. The PC scores or rankings were used to map horses on the same bi-plot. In addition, correlation coefficients of physical measurements and BCS with PC1 and PC2 scores were determined by the CORR procedure (SAS v. 9.4; SAS Institute Inc., Cary, NC) and were used to map these measurements (Table 3.2). The TTEST procedure of SAS 9.4 was used to conduct paired t-test to determine differences among BF measurements. Effects of BCS on BF were analyzed as a completely randomized design in a general linear mixed model with BCS as fixed effect. Analysis of variance was performed by the MIXED procedure of SAS 9.4. Degree of freedom was estimated by Kenward-Roger approximation method. Means, if differing, were separated by a protected t-test. Statistical significance was determined at $P \leq 0.05$.

Results and Discussion

Body Fat Measurements

Principal component analysis indicated that total variance of D_2OF, RFT, RF, and NIR BF variables (TF, LWF, DWF, LWEGF, and DWEGF) can be explained by 2 principal components PC1 (80.93% of total variance) and PC2 (19.07% of total variance). Based on the proximity of these variables, 80.93% of the variance explained by PC1 was primarily contributed by D_2OF and NIR BF variables; whereas the other 19.07% of the variance explained by PC2 was primarily contributed by RF (Fig 3.1). Sources of variance of RF and RFT were identical because RFT was used to calculate RF through simple linear regression.

Correlations between D_2OF and NIR BF variables and between D_2OF and RF had different patterns. Body fat determined by D_2O dilution method was in close proximity to NIR fat measurements, and all were closely related to PC1 ($r = 0.86$ to 0.98; $P < 0.001$; Table 3.1). Body fat predicted by RFT was located further from other fat measurements (Fig 3.1) and was related to both PC1 ($r = 0.60$; $P = 0.008$) and PC2 ($r = 0.79$; $P < 0.001$; Table 3.1), indicating RFT might not be an accurate predictor of BF and needs at least another independent variable that is more correlated to PC1. The Spearman's correlation coefficients (Table 3.2) further validated D_2O dilution as the more accurate predictive measurement of BF. Deuterium oxide dilution was strongly correlated with LWEGF ($r = 0.82$; $P < 0.001$), DWEGF ($r = 0.81$; $P < 0.001$), LWF ($r = 0.76$; $P < 0.001$), DWF ($r = 0.77$; $P < 0.001$); whereas the correlation coefficients between RF and NIR BF measurements were 0.41 ($P = 0.093$), 0.38 ($P = 0.119$), 0.45 ($P = 0.063$), and 0.45 ($P = 0.059$), respectively, which did not indicate a significant correlation. Paired t-test

revealed that D_2O dilution estimated 3.30% less fat than RFT prediction ($P < 0.001$). Both D_2O dilution and RFT prediction overestimated BF by 2.48 to 3.26% ($P < 0.001$) and 5.81 to 6.59% ($P < 0.001$), respectively, compared with dissection and NIR analysis.

Body fat predicted by RFT was not well correlated with NIR analysis of BF. This might be explained by differences in fat deposition in different locations among horses. Westervelt et al. (1976) reported several trials of using RFT ultrasonography to predict BF. The first trial indicated a strong correlation with BF ($r = 0.93$; $P < 0.01$). Among fat thickness of 8 horses at rump, rib, and shoulder areas, that of the rump was greatest and strongly correlated with percentage of ether extractable fat of the empty body (subtracting gut contents; $r = 0.93$). However, the fact that a horse without visible RFT would still be predicted to have 8.64% BF (BF = 8.64 + 4.70 × RFT), which exceeds BF determined by NIR of all horses in the current study, is unlikely. In a separate experiment, the authors used 11 ponies to assess the effect of exercise on fat content and the relationship between RFT and total BF (Westervelt et al., 1976). The prediction equation changed to BF = 3.83 + 5.58 × RFT (Westervelt et al., 1976). These two BF prediction equations for horses and ponies had similar slopes to what was reported by Snedecor and Cochran (1967); however, the intercept in horse prediction equation (8.64%) found by Westervelt et al. (1976) was greater ($P < 0.05$). These authors suggested that further research would be needed to determine the reason for the difference. The current study confirmed that breed type of horses influenced BF predicted by RFT.

The slight overestimation of BF by D_2O dilution as compared with NIR measurements might be partly explained by the assumptions under which D_2O dilution was used to calculate BF, i.e. 4% of isotopic exchange between deuterium hydrogens and

non-water molecules, that fat-free body only includes lean tissues, and that hydration factor of lean tissues is 0.732 (73.2% water in lean tissues). In grass-finished beef separable lean, the hydration factor (water content) is approximately 67 to 73% (USDA, 2017). Applying the maximum value of 0.732 might lead to underestimation of fat-free body percentage (body water divided by hydration factor), thereby overestimating BF (100 – percentage of fat-free body). Moreover, tracer dilution methodology to estimate BF required the determination of body water that diluted D_2O concentration (Speakman et al., 2001b). Therefore, if the D_2O was not sufficiently equilibrated into body water and underestimated, BF prediction could be overestimated. On the contrary, Dugdale et al. (2011b) reported a slight underestimation of BF using the D_2O dilution technique. In the current study, it was assumed that fat from the head, feet (below knees and hocks), hide, tail, visceral tissues (except for separable fat previously mentioned), and bone was minute compared with that of soft tissues and separable internal fat. Brain fat content of lambs, pigs, and cattle consistently ranges from 8.58% to 10.30% (USDA, 2017). Assuming a horse brain weighs 655 g and is 10% fat (521 kg of BW; Rousseeuw and Leroy, 1987), the contribution of the brain to body in the current study would be only 0.005% (in an average of 13.3 kg of TF).

Findings in the current study provided evidence of D_2O dilution being a more accurate BF prediction method for stock-type horses because BF obtained by this method strongly agreed with BF measured through body dissection and NIR analysis. Dugdale et al. (2011b) provided first definitive confirmation of D_2O dilution technique to estimate BF, modeled by dissection and proximate analysis, similar to what was employed in the current study. However, BF data from the current study differed from those reported by

Dugdale et al. (2011a). In the current study, the range of LWF was 1.59% to 7.59%, whereas Dugdale et al. (2011a) reported BF of 1 to 29% in 7 ponies. Narrower range of BF in the current study was likely caused by a closer grouping of BCS and differences in BF deposition between stock-type horses used in the current study and ponies, which more readily accumulate fat than horses. Nonetheless, both studies agreed that D_2O dilution-derived BF was well correlated with BF calculated from proximate analysis of dissected tissues. The authors of the current study and Dugdale et al. (2011a) both approached D_2O dilution method identically and methodically dissected tissues and reconstructed BF from proximate composition. Although D_2O dilution was found to be an accurate, non-invasive method to estimate BF in ponies (Dugdale et al., 2011b), D_2O prediction of body composition in swine indicated that accurate prediction was only applicable among animals with shared physiological resemblances (Rozeboom et al., 1994; Ferrel and Cornelius, 1984). The current study is the first to confirm its accuracy in stock-type horses.

Body Fat and Physical Measurements

Physical measurements, including NC, GC, HT, and BW, were not in close proximity to any fat measurement but were in close to the bi-plot origin (not correlated with either PC1 or PC2; $P \geq 0.57$; Table 3.1), indicating that they might not be accurate predictors of BF. However, BCS and BL were correlated with PC1 ($r = 0.67$, $P = 0.002$; $r = -0.45$, $P = 0.061$; respectively; Table 3.1), indicating that they may be used as BF predictors. Indeed, the Spearman's correlation coefficients of BCS and BL to LWEGF were 0.78 ($P < 0.001$) and -0.44 ($P < 0.065$), respectively (Table 3.1).

Principal component scores were also used to map individual animals onto the bi-plot and visual appraisal indicated that most horses were well clustered by their BCS groups (Fig 3.1), indicating an effect of BCS on BF. Horses of BCS 4 were in close proximity of BL, indicating that BL or similar physical measurements might be needed to predict BF in horses of low BCS. Horses of BCS 6 were in closer proximity to BCS and D_2OF and NIR fat measurements, indicating that BCS might be a strong predictor of BF in horses of greater BCS. Conversely, Dugdale et al. (2011a) found BCS is unlikely to be a sensitive indicator of BF for equids in moderate to obese states. This disagreement might be explained by the differences in body characteristics of horses and ponies. In the current study, only BCS 4, 5, and 6 were used; whereas, the ponies were of BCS 1.25 to 7. Moreover, it is important to recognize the subjective nature of BCS determination and variation in BF in animals of similar BCS, evident by 2 individual animals (BCS 5 and 6) being outliers of BF within BCS 5 and 6 groups, respectively (Fig 3.1).

Effects of BCS on Body Fat

Body condition scores have an effect on BF measured by NIR analysis ($P \leq 0.038$) and BF predicted by D_2O dilution ($P < 0.001$) and RFT ($P = 0.042$; Table 3.3). However, the patterns of impact by BCS on NIR-derived BF were different among the weight bases on which BF was calculated. Horses of BCS 6 were greater than those of both BCS 4 and 5 in LWF (4.67%; $P \leq 0.049$) and DWF (5.16%; $P \leq 0.046$) without differences among horses of BCS 4 and 5 in LWF (2.14% and 3.23%, $P \geq 0.151$) and in DWF (2.40% and 3.62%, $P \geq 0.130$). If the gut content was removed, horses of BCS 6 were greater than those of BCS 4 in LWEGF and DWEGF ($P \leq 0.012$; Table 3.3), with those of BCS 5 being intermediate ($P \geq 0.099$; Table 3.3). The effect of BCS on

estimated values of BF was also different between the 2 prediction methods. Horses of BCS 6 were greatest in D_2OF (10.28%; $P \leq 0.003$), with no difference in D_2OF between those of BCS 4 and 5 (3.60 and 5.99%, respectively; $P = 0.090$). Horses of BCS 6 were greater than those of BCS 5 in RF (10.30 and 9.83%, respectively; $P = 0.014$; Table 3.3); whereas those of BCS 4 were intermediate (9.92%; $P \geq 0.086$).

The variation in patterns of BCS effect on NIR measurements of BF was indicative of the influence of gut content. The BCS had similar effects on LWF and DWF because the only difference between LW and DW was blood weight, which was consistently found at 10.5% of BW (CV = 14.89%; Table 3.4). However, gut content weight varied more by animal (CV = 35.48%; Table 3.4). The effect patterns of BCS on LWF, DWF, and D_2OF were similar, providing further evidence of D_2O dilution being a more accurate predictor of BF than RFT. As previously discussed, univariate analysis further provided evidence that BCS was indicative of varying in BF, which was explained by the fact that BCS determination requires appraisals at multiple anatomical locations, where fat has been reported to accumulate (Henneke et al., 1983); whereas RF prediction relies solely on measurement of 1 location. According to the current study, it is possible to develop a BF prediction method based on visual and palpable appraisals and physical measurements.

Conclusion

It is evident that D_2O dilution is the most accurate, non-invasive method to estimate BF in horses; therefore, D_2OF should be used as future dependent variable in BF prediction models. Rump fat thickness is commonly used, however, is not accurate as the sole predictor of BF and should be used in conjunction with other physical measurement

variables. Body condition score, in contrast with previous studies in ponies, may serve as a valuable predictor of BF in stock-type horses. However a wider range of BCS, rigid scoring standards, and consistent evaluator training are crucial to future modeling and application. The findings also suggested that BL and other physical measurements should be explored as tools to assess total BF. These findings are useful in further research, in which accurately predicting BF is important, such as the development of mathematical nutrition models, determination of requirements for optimized reproductive function, and establishment of welfare thresholds. Should a new prediction model be developed using BCS and physical measurements, horse owners will be able to assess energy status of their horses more accurately.

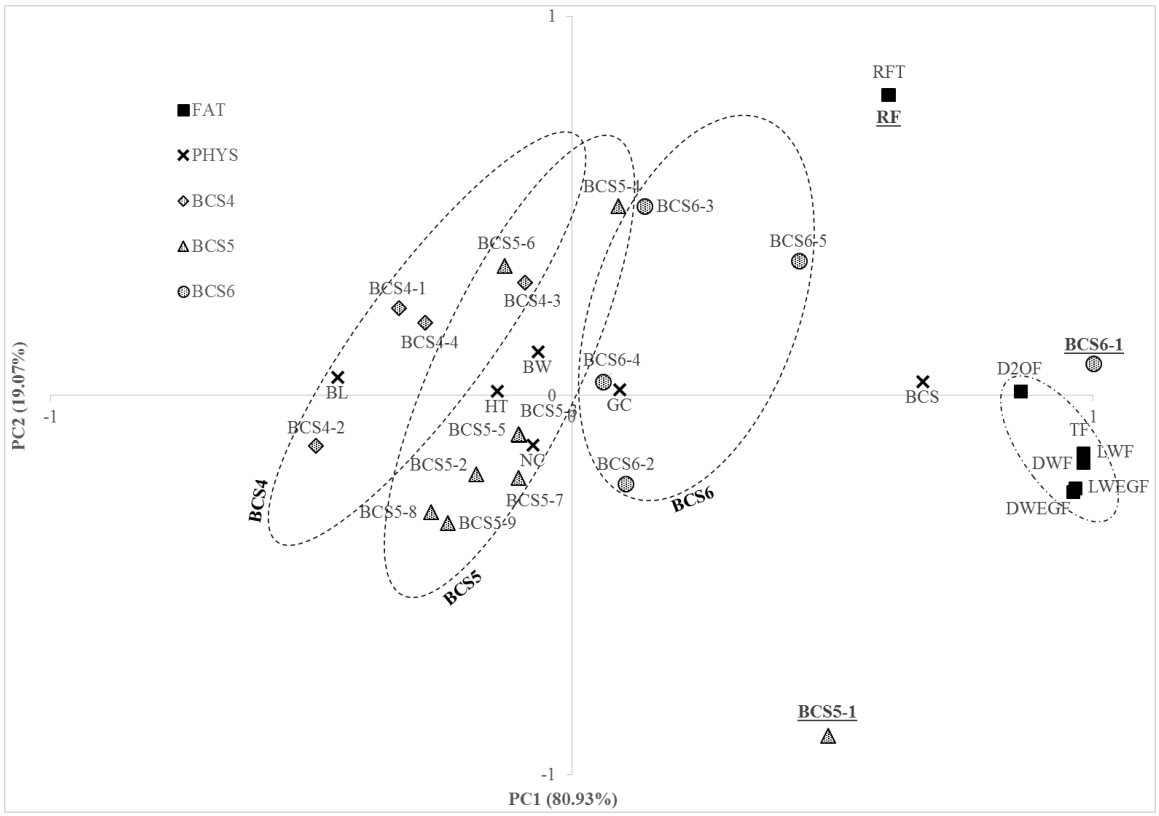

Figure 3.1 Principal component analysis of body fat (BF, %) estimated by rump fat thickness (RFT and RF) and deuterium oxide dilution (D_2OF), BF measured by NIR analysis on live weight (LWF), dead weight (DWF), live weight with empty gut (LWEGF), and dead weight with empty gut (DWEGF) bases, and physical measurements including BCS, body length (BL), height (HT), neck circumference (NC), girth circumference (GC), and BW.

The coordinates are correlation coefficients of BF and physical measurements with PC1 (explaining 80.93% of variance) and PC2 (19.07% of variance) and scores of individual animals.

Table 3.1 Factor loadings1 (correlation coefficients with principal component PC1 and PC2) of body condition score (BCS), body length (BL), neck circumference (NC), girth circumference (GC), height (HT), BW, rump fat thickness (RFT), body fat (BF, %) predicted by D_2O (D_2OF), BF predicted by rump fat thickness (RFT and RF), total tissue fat (TF), BF measured by near infrared spectroscopic analysis (NIR) on a live weight basis (LWF), BF measured by NIR on a dead weight basis (DWF), BF measured by NIR on a live weight, empty gut basis (LWEGF), and BF measured by NIR on a dead weight, empty gut basis (DWEGF)

Variable	PC1 (89.97%)		PC2 (19.03%)	
	r	*P* value	*r*	*P* value
BCS	0.67	0.002	0.04	0.887
BL	-0.45	0.061	0.05	0.849
NC	-0.07	0.769	-0.13	0.606
GC	0.09	0.717	0.02	0.951
HT	-0.14	0.571	0.01	0.967
BW	-0.07	0.798	0.12	0.651
RFT	0.61	0.007	0.79	< 0.001
D_2OF	0.86	< 0.001	0.01	0.966
RF	0.61	0.008	0.79	<.0001
TF	0.98	< 0.001	-0.15	0.550
LWF	0.98	< 0.001	-0.18	0.486
DWF	0.98	< 0.001	-0.18	0.485
LWEGF	0.97	< 0.001	-0.24	0.328
DWEGF	0.96	< 0.001	-0.25	0.311

[1]Based on principal component analysis of RFT, D_2OF, RF, TF, LWF, DWF, LWEGF, and DWEGF and correlation analysis between factor scores and BCS, BL, NC, GC, HT, and BW.

Table 3.2 Spearman's correlation coefficients of deuterium oxide and rump fat thickness predictions of body fat (D_2OF and RF, %; respectively) with near-infrared (NIR) spectroscopic analysis of body fat (BF, %)

NIR BF	D_2O		RF	
	r	*P* value	*r*	*P* value
LWF[1]	0.76	< 0.001	0.45	0.063
DWF[2]	0.77	< 0.001	0.45	0.059
LWEGF[3]	0.82	< 0.001	0.41	0.093
DWEGF[4]	0.81	< 0.001	0.38	0.119

[1]BF based on live weight
[2]BF based on dead weight
[3]BF based on live weight with empty gut
[4]BF based on dead weight with empty gut

Table 3.3 Body fat (BF, %) of horses of varying BCS.

BCS	LWF[1]	DWF[2]	LWEGF[3]	DWEGF[4]	RF[5]	D_2OF[6]
4	2.14[b]	2.40[b]	2.31[b]	2.61[b]	9.92[ab]	3.60[b]
5	3.23[b]	3.62[b]	3.62[ab]	4.10[ab]	9.83[b]	5.99[b]
6	4.67[a]	5.16[a]	4.99[a]	5.56[a]	10.30[a]	10.28[a]

[a,b]Within a column, means without common letters differ ($P \leq 0.05$)
[1]BF based on live weight
[2]BF based on dead weight
[3]BF based on live weight with empty gut
[4]BF based on dead weight with empty gut
[5]BF predicted by rump fat thickness
[6]BF predicted by deuterium oxide dilution

Table 3.4 Body components (% of BW) used to constructed body fat but not dissected for fat recovery.

Body component	Mean	SD[1]	CV[2]
Blood	10.53	1.57	14.89
Esophagus and trachea	0.27	0.16	59.48
Gut content	8.31	2.95	35.48
Full stomach	0.50	0.24	48.71
Empty stomach	0.37	0.15	41.46
Full small intestine	1.81	0.62	34.30
Empty small intestine	0.93	0.31	33.69
Full large intestine	9.68	3.02	31.20
Empty large intestine	3.57	1.67	46.96
Full cecum	2.21	0.73	32.93
Empty cecum	0.86	0.12	13.50
Tail	0.30	0.04	14.92
Hide	2.90	0.30	10.47
Forefeet and hind feet	2.03	0.61	30.18
Reproduction tract	0.24	0.10	40.33
Internal fat	2.15	0.69	32.13
Head	3.74	0.24	6.39

[1]Standard deviation (% of BW)
[2]Coefficient of variation (%)

CHAPTER IV

FATTY ACID COMPOSITION OF MESENTERIC, CARDIAC, SUBCUTANEOUS, INTERMUSCULAR, AND LEAF FAT AND THE RELATIONSHIP BETWEEN FATTY ACID COMPOSITION AND BODY CONDITION SCORE IN HORSES

Introduction

Body condition scores are widely used to estimate overall adiposity in horses (Henneke et al., 1983). Although BCS serves as a useful tool in assessing feeding programs and optimizing reproductive efficiency, it does not account for fatty acid (FA) composition of tissues. Fatty acids are energy-dense molecules serving as an important energy source and integral component of cell membranes. They are stored in adipose tissues as triglycerides and indicate the degree of obesity, and also act as signaling molecules in regulating gene expression, some mechanisms of which remain unknown (Duplus et al., 2000). The proportion of FAs in adipose tissues influence size of adipocytes (Garaulet et al., 2006). Enlargement of cells modifies their metabolic capacity, thus is involved in metabolic complications associated with obesity at the whole body level (LeLay et al., 2001). In humans, n-3 FAs are important for health and disease prevention, including that of coronary heart disease and stroke, inflammation, and possibly behavioral disorders (Connor, 2000). In horses, King et al. (2008) suggested benefits from 20:5n-3 and 22:6n-3 (Portier et al., 2006) could decrease the effects of

exercise-induced hypertension and pulmonary hemorrhage. Furthermore, Vick et al. (2007) provided the first evidence establishing an interrelationship between obesity, inflammatory cytokines, and insulin resistance existed and proposed additional research to describe the nature of these relationships. Thus, research on FA composition of adipose tissues is important for future research on horse health. Therefore, the objectives of the current study are to:

1. Determine the FA composition of mesenteric (MS), cardiac (CD), subcutaneous (SC), intermuscular (IM), and leaf fat (LF) and,

2. To identify relationships between of FA composition and BCS in horses.

Materials and Methods

The current study was conducted under an approved Mississippi State University Institutional Animal Care and Use Committee protocol #15093

Experimental Design and Sample Collection

Twenty-four stock-types horses were selected based on 3 primary criteria: geriatric (≥ 20 years), crippled (defined by the authors as having a chronic limb abnormality inhibiting gait and causing undue discomfort), and/or unsafe (aggressive or combative). All horses were evaluated for BCS and separated into BCS groups of 1 (n = 1), 2 (n = 1), 3 (n = 1), 4 (n = 7), 5 (n = 9), and 6 (n = 5) (Henneke et al., 1983). Horses were maintained on native grass pasture for 4 mo leading up to the current study. Fourteen horses began the current study at desired BCS of 4, 5, or 6, thus continued to be maintained on pasture until slaughter. The remaining 10 horses were fed concentrate (Nutrena Strategy, Nutrena Animal Nutrition, Minneapolis, MN; Cordero et al., 2013) for 30 d in addition to being maintained on the similar pasture until desired BCS was

achieved. Two horses (BCS 1 and 2) failed to meet intended BCS of 3 and were not included in the statistical comparison. At slaughter, BW was recorded to be 433 kg (BCS 1), 415 kg (BCS 2), 376 kg (BCS 3), 468 ± 13 kg (BCS 4), 455 ± 11 kg (BCS 5), and 493 ± 12 kg (BCS 6). Approximately 20 h before slaughter, BCS were confirmed (Henneke et al., 1983) and horses were held in a dry lot overnight with ad libitum access to water. Horses were individually sedated (1.1 mg xylazine/kg BW) and anesthetized (2.2 mg ketamine/kg BW) and KCl solution was administered to cease cardiac function before exsanguination. Euthanized horses were weighed and the head, fore and hind feet, and tail were removed. Carcasses were then eviscerated, dehided, and split at the median plane. Mesenteric, CD, LF, IM, and SC samples were collected from around intestines, around the heart, inside the left flank, in the thoracic region and underneath the latissimus dorsi muscle, and on the surface of semitendinosus muscle, respectively. Connective tissues were removed and fat samples were frozen in liquid nitrogen, pulverized into finely divided powder, and stored at –80°C until subsequent FA analysis.

Fatty Acid Analysis

Fatty acids were extracted from homogenized adipose tissue samples and esterified by using a direct transesterification method (O'Fallon et al., 2007). A 100-mg sample was placed into a 20-mL flat bottom glass vial with PTFE-lined septum (Fischer scientific, Waltham, MA, USA). After methyl tridecanoate (13:0) was added as internal standard (Sigma Aldrich, St. Louis, MO), fat was saponified in presence of potassium hydroxide and methanol. Saponified FAs were then trans-esterified in presence of sulfuric acid into fatty acid methyl esters (FAME). Fatty acid methyl esters were extracted in hexane, transferred into a 2-mL vial, and stored in -20°C freezer until being

determined by gas chromatography. Fatty acid methyl esters were separated and quantified in a GC system (Agilent Technologies, Santa Clara, CA) equipped with an HP- 88 capillary column (30 m × 0.25 mm i.d. × 0.2 μm film thickness; Supelco Inc., Bellefonte, PA) and a flame-ionization detector. Hydrogen was used as carrier gas at 1.5 mL/min and the total run time was 20.83 min. Peaks were identified by FAME standards in Supelco® 37 Component FAME Mix (Sigma-Aldrich, St. Louis, MO), FAME #21 Mix (Restek, Bellefonte, PA), and a customized 17-component FAME mix (Nu-Chek-Prep, Elysian, MN) and quantified by an internal standard calibration method. Concentration (mg/1 g of adipose tissue) of each FA was converted from the corresponding FAME concentration. Percentage of each FA was calculated by dividing its concentration (mg/g) by total FA concentration (mg/g) and then multiplying by 100. Saturation index (SI) was calculated by the ratio of SFA and the sum of MUFA and PUFA, whereas P/S was the ratio between PUFA and SFA.

Statistical Analysis

Horses with total FA concentration of less than 400 mg/g of adipose tissue were excluded because of possible processing errors that might lead to too much muscle and connective tissue contamination. In addition, horses of BCS of 1, 2, and 3 were not used for statistical analysis purposes because there was only 1 horse per BCS. A total of 19 horses was used for MS, CD, LF, and IM (n = 5 for BCS 4, n = 9 for BCS 5, and n = 5 for BCS 6), whereas a total of 17 horses was used for SC (n = 4 for BCS 4, n = 8 for BCS 5, and n = 5 for BCS 6). Predominance order of FA and FA category among and within BCS and fat depot was analyzed as a split-split-plot design with BCS being main factor and horse being main plot, fat depot being split factor and fat sample being split-plot, and

FA or FA category being split-split factor and percentage of FA or FA category being split-split plot, respectively. A general linear mixed model was used with BCS, fat depot, FA or FA category, and their interactions being fixed effects. Effects of BCS on FA composition within each fat depot was analyzed separately as a completely randomized design in a generalized linear mixed model with BCS serving as the only fixed effect. The analysis of variances was performed by the MIXED (split-split plot design) and the GLIMMIX (completely randomized design) procedures of SAS 9.4 (SAS Institute, Cary, NC). Degree of freedom was calculated by Kenward-Roger approximation method. Means, if differing, were separated by the protected t-test in the LSMEANS statement of SAS. Statistical significance was determined at $P \leq 0.05$ unless otherwise noted.

Results

Overview of FA Composition in Fat Depots and BCS

The FA compositions of horses were categorized into SFA, MUFA, and PUFA. Across all depots and BCS, the predominance of individual FAs within a category was similar (Tables 4.1, 4.2, 4.3, 4.4, 4.5). There were 2-way interactions of BCS × FA ($P < 0.001$) and fat depot × FA ($P < 0.001$) but not 3-way interaction of BCS × fat depot × FA ($P = 0.999$), indicating that influence of either BCS or fat depot was independent of each other. The effects of BCS on individual FA and FA categories within each depot were discussed separately.

Palmitic acid (16:0) and stearic acid (18:0) were the greatest and the second greatest FAs ($P < 0.001$) in SFA category, respectively, so were 18:1n-9 and 16:1 within MUFA, and were 18:3n-3 and 18:2n-6 within PUFA category. In general, the major FA were palmitic acid (16:0; 24.85%), stearic acid (18:0; 5.54%), oleic acid (18:1n-9 cis;

27.34%), linoleic acid (18:2n-6; 11.20%), and linolenic acid (18:3n-3; 17.95%). In MS, LF, IM, and SC, 18:1n-9 cis was the predominant FA (28.7 to 29.1%; $P < 0.001$). Cardiac FA composition was an exception with 16:0 being greatest (26.0%; $P < 0.001$). In all other depots, 16:0 was the second greatest FA (23.9% to 25.1%; $P < 0.001$). Linolenic acid was the third greatest FA (16.5 to 22.0%; $P < 0.001$). The predominance of FA showed different patterns within each BCS. In horses of BCS 4, SFA were greater ($P < 0.001$), followed by MUFA and PUFA in the same percentage ($P < 0.83$; Fig 1). Percentage of SFA in horses of BCS 5 was also greater than those of MUFA and PUFA ($P < 0.001$), which also differed ($P < 0.001$; Fig 1) with PUFA percentage being the least ($P < 0.001$). Percentages of SFA and MUFA in horses of BCS 6 were similar ($P < 0.280$); both were greater than PUFA percentage ($P < 0.001$).

The predominance of FA categories was similar in MS, IM, and SC, with PUFA percentage being smallest ($P < 0.001$) but SFA and MUFA percentages being similar ($P \geq 0.505$). An exception was found in CD, where SFA and PUFA percentages (36.81 and 36.42%, respectively) did not differ ($P = 0.740$), and MUFA percentage was less than both (26.77%; $P < 0.001$). Moreover, in LF, percentages of SFA (37.60%), MUFA (32.72%), and PUFA (29.69%) all differed ($P \leq 0.008$). When comparing across fat depots, LF had more SFA (2.85%, $P = 0.014$) than MS and more MUFA (5.95%; $P < 0.001$) than CD. Intermuscular fat, MS, and SC had more MUFA than LF and CD ($P \leq 0.048$). Cardiac adipose tissue had the greatest PUFA percentage (36.42%; $P < 0.001$); whereas it was similar among other depots ($P > 0.050$).

There were overall effects of BCS and fat depot on SI ($P \leq 0.006$) and P/S ratio ($P \leq 0.029$). Across all BCS, CD had a greater SI (0.58; $P = 0.010$) than MS (0.53).

Likewise, the SI for LF (0.60) was greater than IM (0.56; $P = 0.015$), MS (0.53; $P < 0.003$), and SC (0.55; $P = 0.009$). Across fat depots, horses of BCS 5 had greater SI (0.59) than 6 (0.55; $P = 0.002$), and BCS 4 was intermediate (0.56; $P \geq 0.057$). Compared to all other fat depots, CD had the greatest P/S ratio (1.00; $P \leq 0.031$). There was no difference in P/S ratio observed between any other depots ($P \geq 0.113$). Across fat depots, horses of BCS 4 had greater P/S ratio (0.89) than 5 (0.79; $P = 0.015$), and BCS 6 was intermediate (0.87; $P \geq 0.060$).

Effects of BCS in Cardiac Adipose Tissues

The percentages of most FA in CD were not affected by BCS ($P \geq 0.060$), except that of heptadecanoic acid (17:1n-8) and 18:0 (Fig 4.3). The percentage of 17:1n-8 in horses of BCS 6 (0.65%) was intermediate ($P \geq 0.078$;), whereas those in horses of BCS 5 (0.68%) and 4 (0.57%) differed ($P = 0.008$; Table 4.1). The percentage of 18:0 was intermediate in horses of BCS 4 (5.83%; $P \geq 0.057$); but those of BCS 5 (6.38%) and 6 (4.69%) differed ($P = 0.003$; Table 4.1). The percentages of SFA, MUFA, and PUFA were not affected by BCS ($P \geq 0.144$; Table 4.1). Similarly, the percentages of predominant FA, including 14:0 (3.48 to 3.67%), 16:0 (25.4 to 26.48%), 16:1n-7 (2.33 to 3.06%), 18:1n-9 (21.74 to 23.98%), 18:2n-6 (12.72 to 13.11%), and 18:3n-3 (19.88 to 23.63%) were not influenced by BCS ($P \geq 0.107$; Table 4.1).

Effects of BCS in Intermuscular Adipose Tissues

In IM, the percentages of capric acid (10:0), margaric acid (17:0), 18:0, 16:1n-7, 17:1n-8, and trans linoleic acid (18:2 trans) were affected by BCS ($P \leq 0.05$; Table 4.2). The percentage of 10:0 was greatest in horses of BCS 4 (0.07%; $P \leq 0.040$), whereas

BCS 5 (0.05%) and 6 (0.04%) were not different ($P = 0.191$). The percentage of 17:0 was intermediate in horses of BCS 4 (0.62%; $P \geq 0.065$); but those of BCS 5 (0.68%) and 6 (0.49%) differed ($P = 0.004$). Percentage of 18:0 was greater ($P \leq 0.025$) in horses of BCS 4 and 5 (5.45% and 5.36%, respectively) than those of BCS 6 (3.88%; Fig 4.6). In BCS 6, percentage of 16:1n-7 was greatest (6.45%; $P \leq 0.065$) compared with BCS 4 (4.45%) and 5 (5.14%), between which there was no difference ($P = 0.338$). The percentage of 17:1n-8 in horses of BCS 4 (0.82%) and 6 (0.88%) were similar ($P = 0.302$), whereas the percentage in horses of BCS 5 was greater than both (0.98%; $P \leq 0.040$). Percentage of 18:2 trans was greater ($P \leq 0.036$) in horses of BCS 4 (0.04%) and 5 (0.04%), than BCS 6 (0.03%). The average percentages of SFA, MUFA, and PUFA were 35.5%, 36%, and 28%, respectively and were not affected by BCS ($P \geq 0.118$; Table 4.2). Similarly, the percentages of predominant FAs 14:0 (4.45 to 4.53%), 16:0 (24.23 to 25.45%), 18:1n-9(28.08 to 29.52%), 18:2n-6 (9.40 to 11.71%), and 18:3n-3 (15.47 to 17.48%) were not affected by BCS ($P \geq 0.107$; Table 4.2).

Effects of BCS in Subcutaneous Adipose Tissues

The percentages of FAs in SC were not affected by BCS ($P \geq 0.060$), except that of 10:0 (Table 4.3). The percentage of 10:0 was greater ($P = 0.014$) in horses of BCS 4 (0.060%) than BCS 6 (0.04%), whereas BCS 5 was intermediate (0.05%; $P \geq 0.114$). The average percentages of SFA, MUFA, and PUFA were 35.5%, 36%, and 28%, respectively, and were not affected by BCS ($P \geq 0.464$; Table 4.3). Similarly, the percentages of predominant fatty acids, 14:0 (4.35 to 4.73%), 16:0 (23.92 to 24.73%), 18:0 (4.32 to 5.59%), 16:1n-7 (4.72 to 6.41%), 18:1n-9 (28.10 to 29.78%), 18:2n-6 (9.49

to 11.37%), and 18:3n-3 (15.61 to 17.18%) were not influenced by BCS ($P \geq 0.107$ Table 4.3; Fig 4.9, 4.10, 4.11).

Effects of BCS in Leaf Fat

In LF, few differences between FA percentages among horses of BCS 4, 5, and 6 were observed. The majority of differences among BCS were seen in percentages of FAs present in relatively small amounts. Percentage of palmitoleic acid (16:1n-7) was greatest in horses of BCS 6 (4.02%; $P \leq 0.05$), however there were no differences between horses of BCS 4 and 5 (2.94% and 3.30%, respectively; $P \leq 0.331$; Fig 4.13). Stearic acid (18:0) ranged from 5.63% to 7.10% with horses of BCS 6 having the smallest percentage ($P \leq 0.017$), and no statistical differences being observed between horses of BCS 4 and 5 ($P \leq 0.539$; Fig 4.12). Percentages of margaric acid (17:0) and heptadecanoic acid (17:1n-8) also differed, with horses of BCS 6 having the smallest percentage of 17:0 (0.65%; $P \leq 0.010$) and horses of BCS 4 having the smallest percentage of 17:1 n8 (0.72%; $P \leq 0.009$; Table 4.4). There was no difference in percentage of 17:0 between BCS 4 and 5 ($P \leq 0.222$) or percentage of 17:1 n8 between BCS 5 and 6 ($P \leq 0.219$; Table 4.4). No difference was observed between BCS in percentages of all other major FA including myristic acid (14:0; $P \leq 0.826$), palmitic acid (16:0; $P \leq 0.623$), oleic acid (18:1 n9 cis; $P \leq 0.254$), linoleic acid (18:2 n6; $P \leq 0.099$), linolenic acid (18:3 n3; $P \leq 0.583$), and arachidonic acid (20:4 n6; $P \leq 0.085$). Additionally, there was no difference in percentages of total SFA ($P \leq 0.487$), MUFA ($P \leq 0.241$), or PUFA ($P \leq 0.392$) among horses of BCS 4, 5, and 6 (Table 4.4).

Effects of BCS in Mesenteric Adipose Tissues

Data from MS was similar to LF in that few FA categories differed among horses of BCS 4, 5, and 6. In MS, the major FAs in which there were differences among BCS included 16:1n-7 ($P \leq 0.014$; Fig 4.16) and 18:0 ($P \leq 0.007$; Fig 4.15). Horses of BCS 6 had the greatest percentage of 16:1n-7 (4.69%) compared to BCS 4 and 5 ($P \leq 0.004$; $P \leq 0.030$, respectively; Table 4.5). Percentages of 18:0 were less in horses of BCS 6 (4.56%) than those of BCS 4 and 5 ($P \leq 0.013$; $P \leq 0.003$, respectively; Table 4.5). No differences were observed among BCS in other major FA including 14:0 ($P \leq 0.592$), 14:1 n5 ($P \leq 0.627$), 16:0 ($P \leq 0.999$), 18:1 n9 cis ($P \leq 0.350$), 18:3 n3 ($P \leq 0.849$), and 20:4 n6 ($P \leq 0.227$; Table 4.5). There was also no difference in SFA ($P \leq 0.249$), MUFA ($P \leq 0.145$), or PUFA ($P \leq 0.408$) among BCS (Fig 4.15, 4.16, and 4.17).

Discussion

Overall, equine adipose tissue FA composition appears to be more greatly impacted by fat depot than BCS. Bjørndal et al. (2011) stated that the distribution of FA among fat depots seems to be more important in the development of obesity-related health issues than total adipose tissue mass. In humans, metabolic complications related to obesity are closely associated with abdominal rather than peripheral fat deposition (Smith et al., 2001). These authors suggested that health issues caused by obesity were largely caused by an accumulation of excessive visceral and midsection subcutaneous adipose tissues (Smith et al., 2001). The functional differences between visceral and subcutaneous adipocytes are likely related to their anatomical location, where in visceral depots, macrophages produce more inflammatory cytokines and less adiponectin (Hamdy et al., 2006). Epicardial fat is another visceral depot that has received an increasing

amount of attention in recent years. According to Iacobellis et al. (2005), epicardial fat is a metabolically active tissue that secretes several bioactive molecules including adiponectin, resistin, and inflammatory cytokines that may influence cardiac function. In a healthy human, epicardial and perivascular fat depots may alter vascular functions and energy partitioning for protective purposes, however excessive accumulation of such depots results in lipotoxic, prothrombotic, and proinflammatory effects (Iozzo, 2011).

In humans, certain FAs may be more detrimental to insulin activity than others (Lovejoy et al., 2002). Storlien et al. (1991, 1993) found high intakes of certain PUFAs and SFA induce severe insulin resistance in rats, whereas MUFAs and n-3 FAs had less deleterious effects. Omega-3 FAs, also called n-3 FAs, are PUFAs that are suggested to play a wide array of roles in various physiological pathways and in prevention/improvement of several diseases (Deckelbaum et al., 2006). Of the 3 major n-3 FAs, 18:3n-3 (16.30 to 21.54%), 20:5n-3 (0.03 to 0.05%), and 22:6n-3 (0.02 to 0.04%), only 18:3n-3 was present in appreciable amounts in the current study, being the third greatest in percentage across all depots. This is likely due to the inefficiency in which 20:5n-3 and 22:6n-3 are synthesized from their 18:3 precursor; therefore, maximizing the availability of these FAs to the tissues depends largely on dietary delivering (Deckelbaum et al., 2006). In general, the health benefits associated with n-3 FAs are linked to inhibition or alteration of eicosanoid pathways, which may reduce inflammatory responses; adjustment of enzymes or molecules that are important in various signaling pathways that involve normal and pathogenic cell function; assimilation of n-3 FAs into cell membranes; and direct influence on gene expression (Deckelbaum et al., 2006). In the current study, the depot with the greatest percentage of 18:3n-3 was CD, with MS

being greater than IM, and LF and SC as intermediates. The greater percentage of health benefitting 18:3n-3 in CD may be related to the depot's protective purposes, in contrast to IM, which mainly serves as storage of triglycerides.

In most mammalian species, de novo FA synthesis occurs predominately in the liver or adipose tissues. According to Ingle et al. (1972), tissue capacity for lipogenesis is location dependent. For example, lipogenic capacity of adipose tissue appears to be greatest in the internal fat depots of lambs and calves, however subcutaneous fat tended to be more active in mature sheep and bullocks (Ingle et al., 1972). In humans, the synthesis of long-chain FAs was reported to be 10 to 20 times greater in omental versus subcutaneous adipose tissues when tested with acetate-1-C^{14} and about 2 times greater when tested with glucose-C^{14} (Hamosh et al., 1963). This is illustrated in the current study where the general pattern of results indicated greater percentages of long-chain FAs, 18:2n-6, 20:0, 18:3n-3, 20:4n-6, 22:1n-9, and 20:5n-3, in internal fat depots compared to SC. Suagee et al. (2010) examined substrate utilization and sites of FA synthesis in the horse. They found that the majority of de novo lipogenesis in the horse takes place in adipose tissue, with MS and SC depots showing markedly greater lipogenic activity than hepatic tissue assessed through in vitro incubation. Furthermore, MS exhibited greater substrate incorporation into lipids than SC. Additionally, these authors found substrate preference to vary, with nutrient availability to be a likely determining factor. They noted that although glucose and acetate derived ^{14}C labels were equally present in the fat, suggesting that the 2 substrates were equally used in FA synthesis, low ATP-citrate lyase activity in the adipose tissue indicated that acetate was the predominant precursor for FA synthesis in the horse. This is consistent with findings in ruminant

animals (Ingle et al., 1972). The greater use of acetate as a lipogenic substrate in herbivores is likely due to the low availability of dietary glucose and greater production of VFAs via fermentation (Suagee et al., 2010). The results pertaining to the third objective of Suagee et al. (2010) showed that the majority of NADPH production coming from glucose oxidation through the pentose phosphate pathway. While there were both similarities and differences between the lipogenic activity of equine MS and SC fat depots found in the aforementioned study and that of ruminant animals (Ingle et al., 1972), Suagee et al. (2010) proposed that lipogenic capacity of equine fat depots may be more similar to that of young, growing meat animals that are still accumulating fat viscerally as opposed to meat animals closer to market weight that are fed to achieve greater overall and intramuscular fat.

Robb et al. (1972) collected adipose tissue samples from the mesentery, the crest area of the neck, and subcutaneous depots covering the ribs in 3 horses, from perinephric depots in 2 horses, and subcutaneous depots around the abdomen of 1 horse. Percentages for 16:1 (7.43%) and 18:2n-6 (20.36%) in mesenteric adipose tissue were greater, and 18:3n-3 (4.73%) were smaller compared to corresponding data from the current study. In the rib and abdomen subcutaneous depots, 16:0 (27.97%),18:1 (31.67%), and 18:2n-6 (17.11%) were found in smaller percentages, and 18:3n-3 (4.36%) in greater percentages than the results indicate for the current study. In the abdomen subcutaneous depots, 16:0 (27.80%), 18:1 (33.26%), and 18:2n-6 (12.76%) were also found in smaller percentages, and 18:3n-3 (10.21%) in greater percentages than the results indicate for the current study. Overall, the authors concluded that the FA composition of the observed adipose tissues were similar, but varied greatly between individual animals. The results pertaining

to the comparison between adipose tissues were similar to the current study when only MS and SC depots are taken into account, which were similar in FA and FA category predominance. However, in the current study, there were no differences observed in MS and SC FA composition between BCS, contrasting the reported differences between individual horses by Robb et al. (1972).

There were few consistent patterns of BCS influence on FA composition. Few differences were found for SFA, MUFA, and PUFA categories within each fat depot, which could be attributed to similar percentages of predominant FA in each category and only slight differences in others among BCS. Overall, the proportions of total SFA, MUFA, and PUFA in equine adipose tissue are relatively different from those in adipose tissues of other livestock species. Fat in cattle has much greater proportions of SFA (44.39 to 47.84%) and MUFA (46.83 to 47.81%) than that of PUFA (5.14 to 8.77%; Dinh et al., 2010). The percentage of SFA in swine fat is about 38.3%, MUFA is about 38.2%, and PUFA is about 15.9% (Enser et al., 1996). The smaller proportion of SFA in equine adipose tissue compared to fat of ruminants can be attributed to the fact that horses are monogastric animals mainly grazed on grass, and therefore, are unable to hydrogenate PUFA to SFA in the rumen as do cattle and other ruminants (Robb et al., 1972). Rumen microbes modify consumed dietary lipids by rapidly hydrolyzing them from their esterified form to free FA and glycerol. Lipolysis is a necessary initial step before biohydrogenation can occur due to its dependence on free carboxyl compound. The extent to which hydrolysis occurs in the rumen is dependent on the nature of the lipids. Plant oils are more completely hydrolyzed (over 90%) than fish oils (less than 50%).

Being monogastric animals, horses are more susceptible to changes in FA composition of adipose tissue from the oils in their diet compared to ruminants. In pigs, feeding great concentrations of 18:2n-6, a FA common in grains and oil seeds, yields notably increased proportions of this FA in the tissues (Wood et al., 2008). Other dietary fats containing specific FAs can influence tissue FA composition. Teye et al. (2006a, 2006b) fed 3 concentrate diets containing 2.8% added oil from various sources: palm kernel oil (high in 12:0, 14:0, and 18:0), palm oil (high in 16:0 and 16:1), and soybean oil (high in 18:2n-6). Their results indicated that the proportions of 12:0, 14:0, and 18:2n-6 in the adipose and muscle tissues were most greatly impacted by dietary sources of fat. However, dietary fat did not have a significant influence on either monounsaturated or saturated C16 and C18 FAs (Teye et al., 2006a, 2006b). This can be explained by the fact that, in pigs, 12:0 and 14:0 are mostly supplied by the diet, and 18:2n-6 is entirely derived from the diet; whereas larger proportions of C16 and C18 monounsaturated and saturated FAs are a result of de novo synthesis by adipose tissues, thus limiting the impact of dietary supplements (Wood et al., 2008). Furthermore, Cameron et al. (2000) found nutritional effects on fat characteristics in pigs to be stronger than genetic effects, stating that nutritional approaches will allow for a decreased n-6:n-3 FA ratio, thus improving the nutritional quality of pork in human health. The major FAs that comprise grass pasture include 16:0 (15.9%), 18:2 (13.2%), and 18:3 (61.3%; Palmquist and Jenkins, 1980), thus it would be expected that the adipose tissue FA composition of horses maintained on native grass pasture would be similar. Results of the current study do reflect this as 16:0, 18:3n-3, and 18:2n-6 were among the major FAs found in the adipose tissues. However, 18:1n-9 cis was found in the greatest percentage in all fat

depots except for CD, whereas Palmquist and Jenkins (1980) found grass pasture to have only 3.4 % 18:1. This is likely due to a large amount of 18:1n-9 cis being a result of de novo synthesis in the adipose tissues from desaturation of 18:0 by Δ9-desaturase activity. Unfortunately, dietary intakes of the horses used in the current study varied and were not recorded in detail; therefore, further speculation of differences in FA composition due to dietary intake is not possible.

Literature pertaining to the impact of BCS on FA composition of adipose tissue in livestock species is sparse. Lake et al. (2007) reported overall concentrations of FA to be greater in lactating beef cows of BCS 6 than 4 and found greater percentages of 18:2n-6 and 18:0 in adipose tissue of cows of BCS 4 compared to 6, however other significant impacts of BCS on FA percentages were not noted. In the current study, there were no differences in the percentage of 18:2-n6 among horses in different BCS. Contrary to results of Lake et al. (2007), horses of BCS 6 had greater percentages of 18:0 than BCS 4 in MS, IM, and LF depots.

Conclusion

In the current study, the more influential factor in FA composition of equine adipose tissue was fat depot when compared to BCS. Therefore, further characterization of various fat depots, including the identification of roles and health effects of important FA, may aid future discoveries in the etiology of common equine metabolic issues such as Equine Metabolic Syndrome, Cushing's Disease, and Equine Hyperlipemia. Monogastric animals incorporate more dietary FAs into tissues than ruminants; therefore researching FA supplementation or restriction regimes to improve equine health and

production is warranted. Lastly it is necessary to use wider range of BCS to investigate the effect of overall adiposity on FA composition in horses.

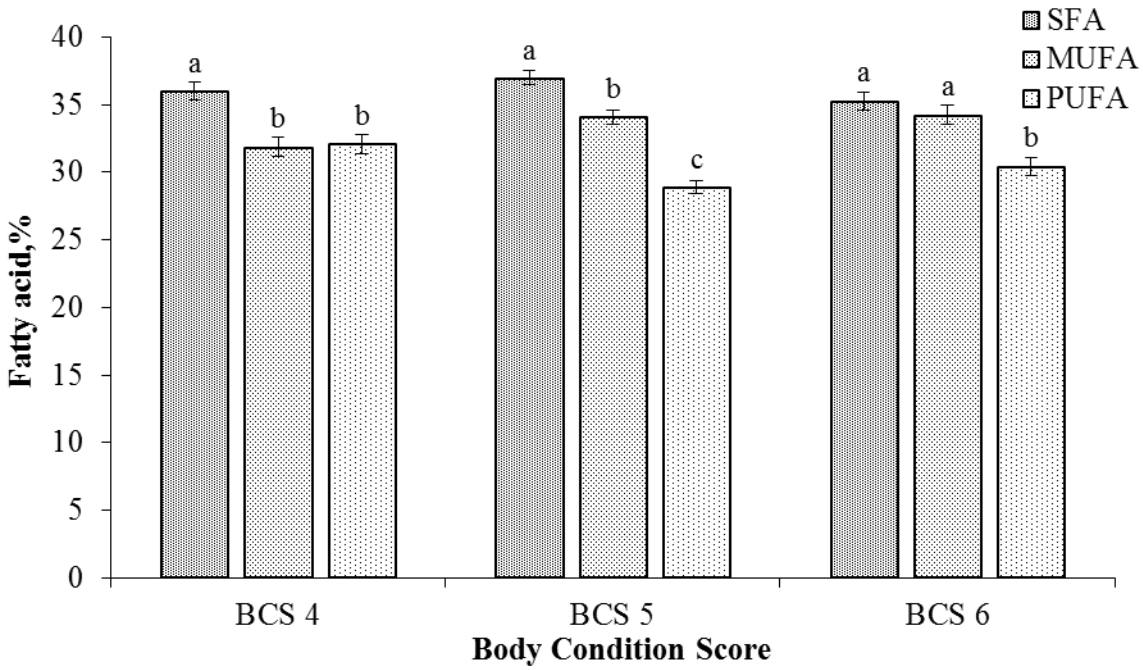

Figure 4.1 Percentages of SFA, MUFA, and PUFA in horses of body condition scores (BCS) 4 (n = 5), 5 (n = 9), and 6 (n = 5).

[a,b,c]Within a BCS, means without common letters differ ($P \leq 0.05$)

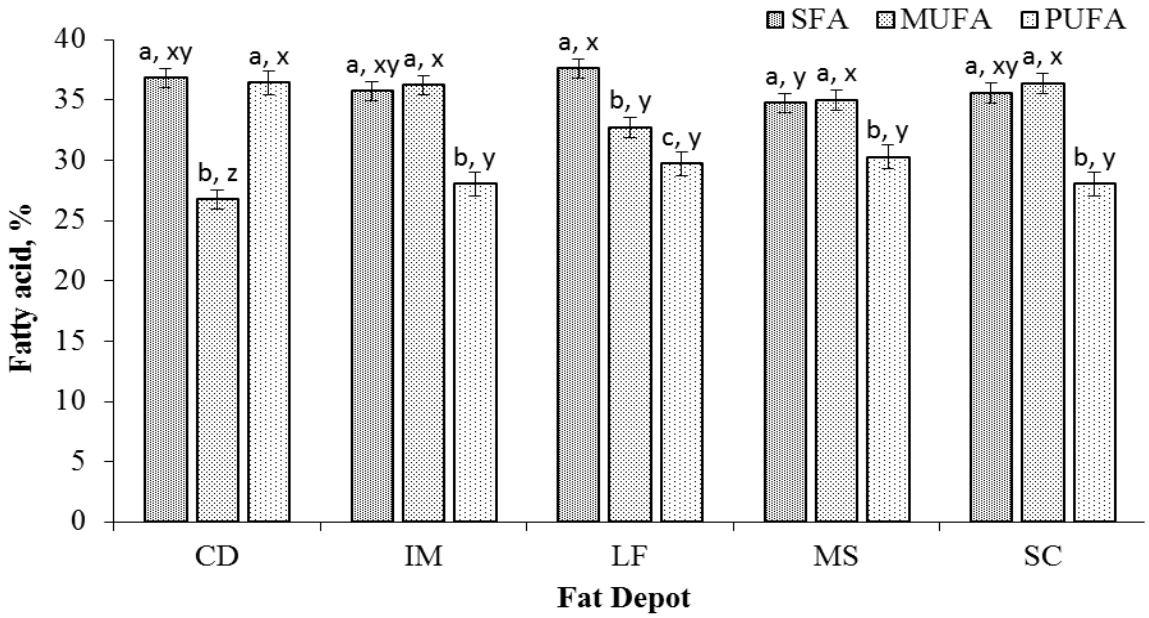

Figure 4.2 Percentages of SFA, MUFA, and PUFA in cardiac (CD), intermuscular (IM), leaf fat (LF), mesenteric (MS), and subcutaneous (SC) adipose tissues.

[a,b,c]Within a fat depot, means without common letters differ ($P \leq 0.05$)
[x,y,z]Within a FA category, means without common letters differ ($P \leq 0.05$)

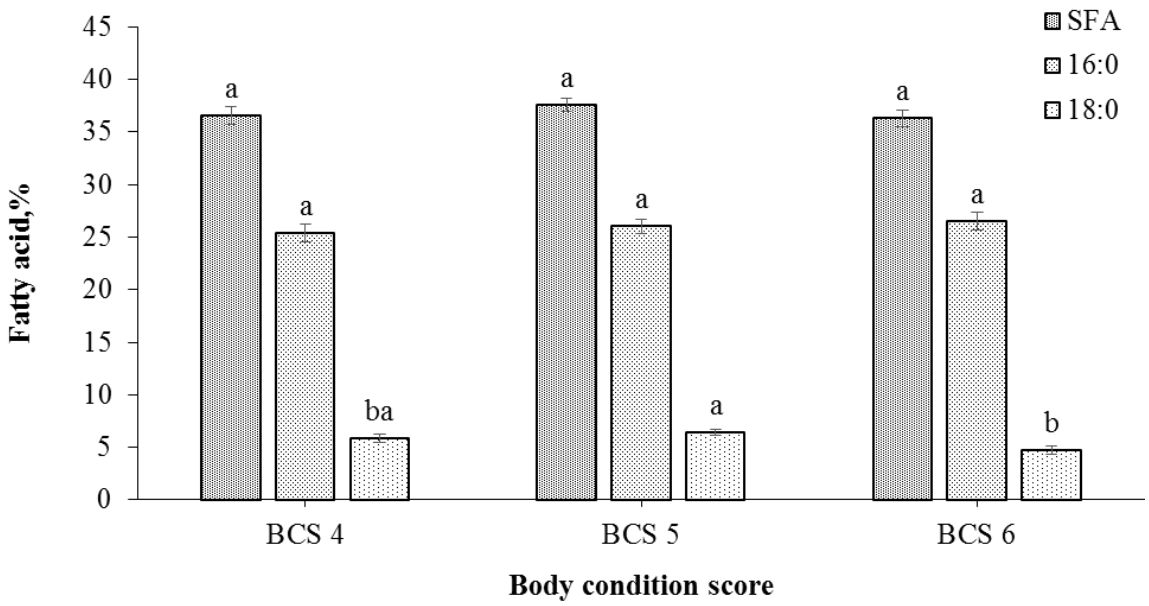

Figure 4.3 Percentage of total saturated fatty acid (SFA), 16:0, and 18:0 FAs in cardiac adipose tissue of horses in BCS 4 (n = 5), 5 (n = 9), and 6 (n = 5).

[abc]Within each category of FA, means without common letters differ ($P \leq 0.05$)

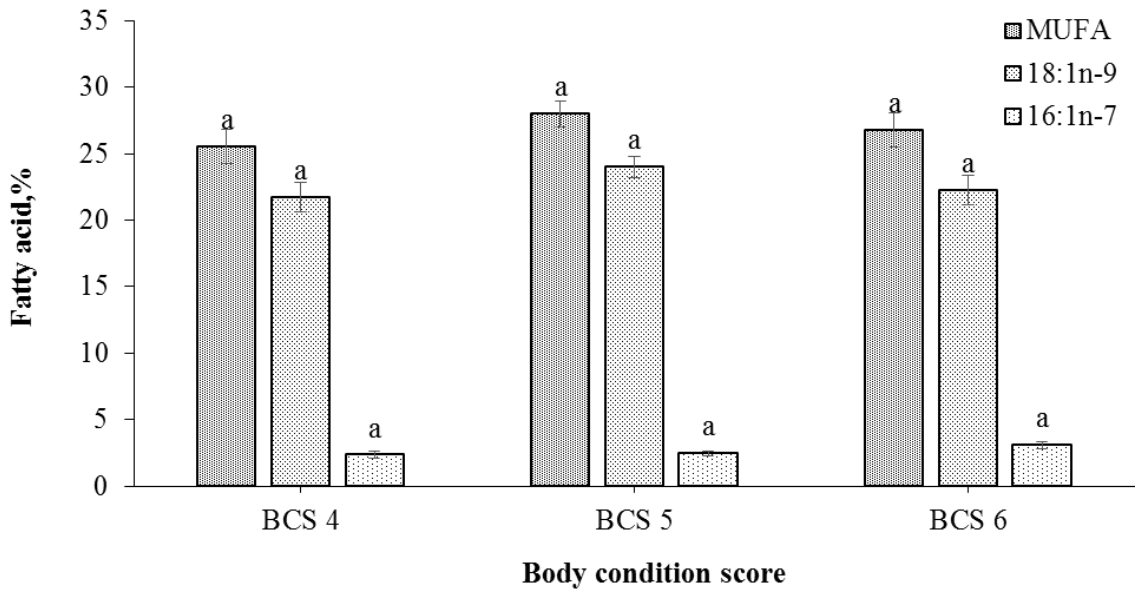

Figure 4.4 Percentage of total monounsaturated fatty acids (MUFA), 16:1, and 18:1 FAs in cardiac adipose tissue of horses in BCS 4 (n = 5), 5 (n = 9), and 6 (n = 5).

[abc]Within each category of FA, means without common letters differ ($P \leq 0.05$)

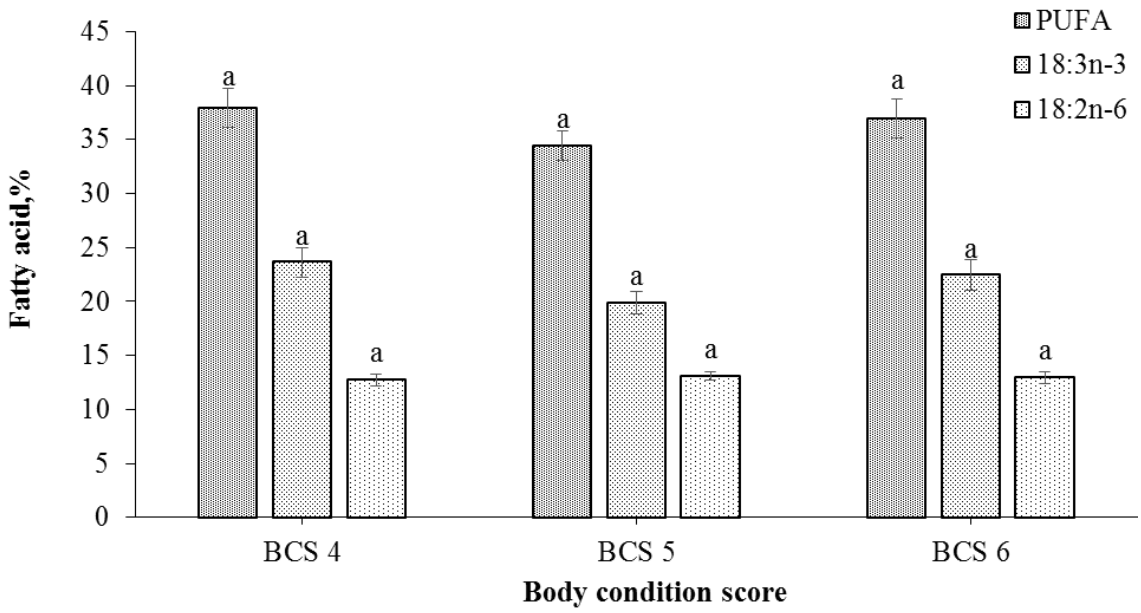

Figure 4.5 Percentage of total polyunsaturated fatty acids (PUFA), 18:2, and 18:3 FAs in cardiac adipose tissue of horses in BCS 4 (n = 5), 5 (n = 9), and 6 (n = 5).

[abc]Within each category of FA, means without common letters differ ($P \leq 0.05$)

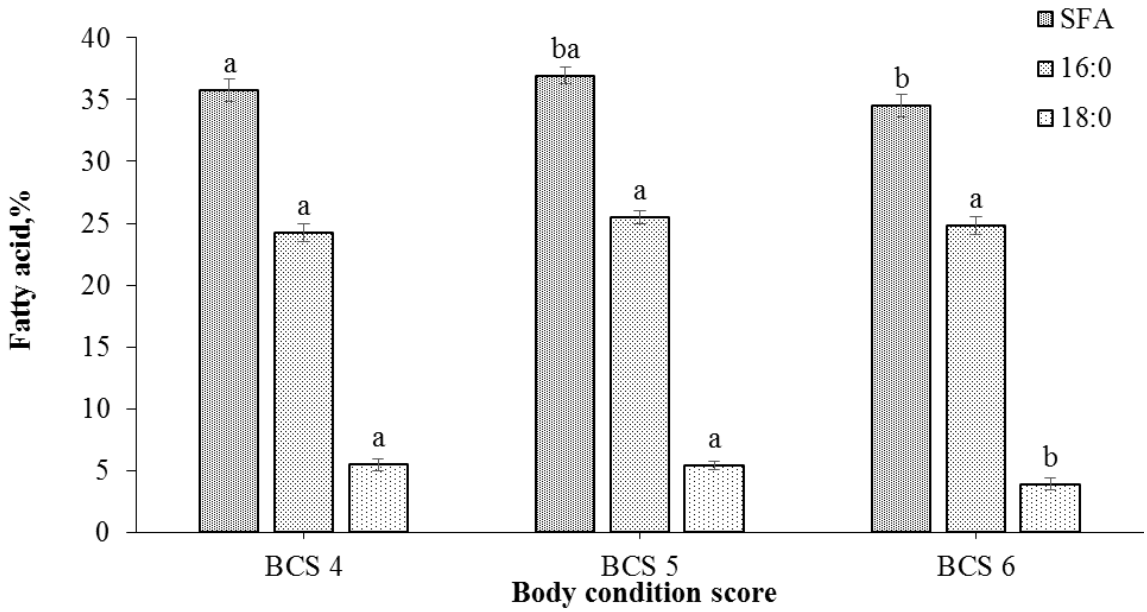

Figure 4.6 Percentage of total saturated fatty acid (SFA), 16:0, and 18:0 FAs in intermuscular adipose tissue of horses in BCS 4 (n = 5), 5 (n = 9), and 6 (n = 5).

[abc]Within each category of FA, means without common letters differ ($P \leq 0.05$)

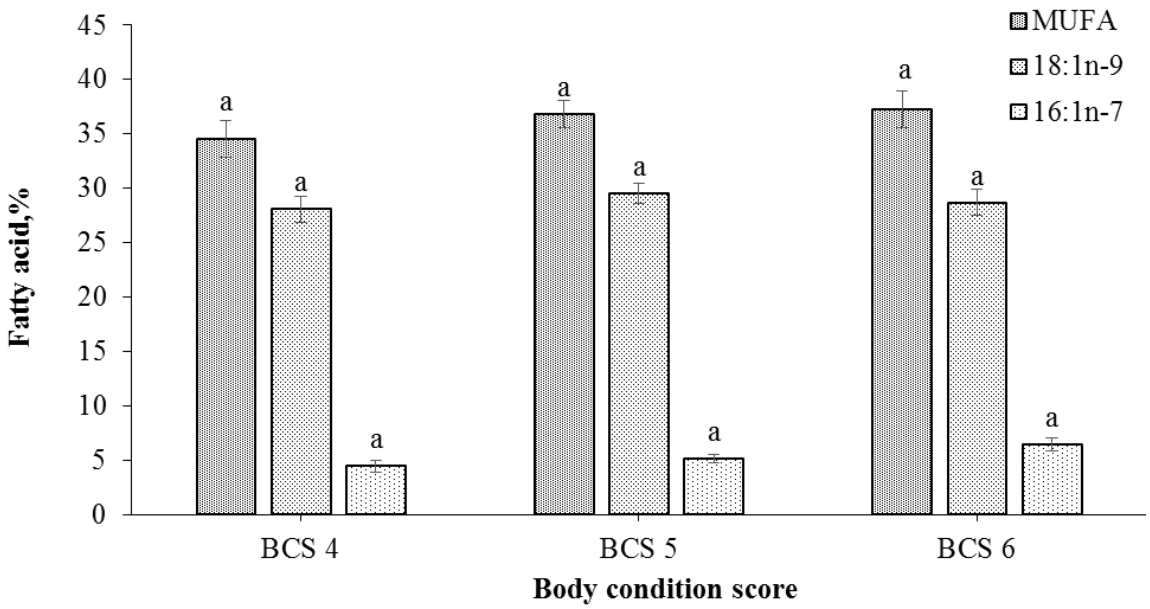

Figure 4.7 Percentage of total monounsaturated fatty acids (MUFA), 16:1, and 18:1 FAs in intermuscular adipose tissue of horses in BCS 4 (n = 5), 5 (n = 9), and 6 (n = 5).

[abc]Within each category of FA, means without common letters differ ($P \leq 0.05$)

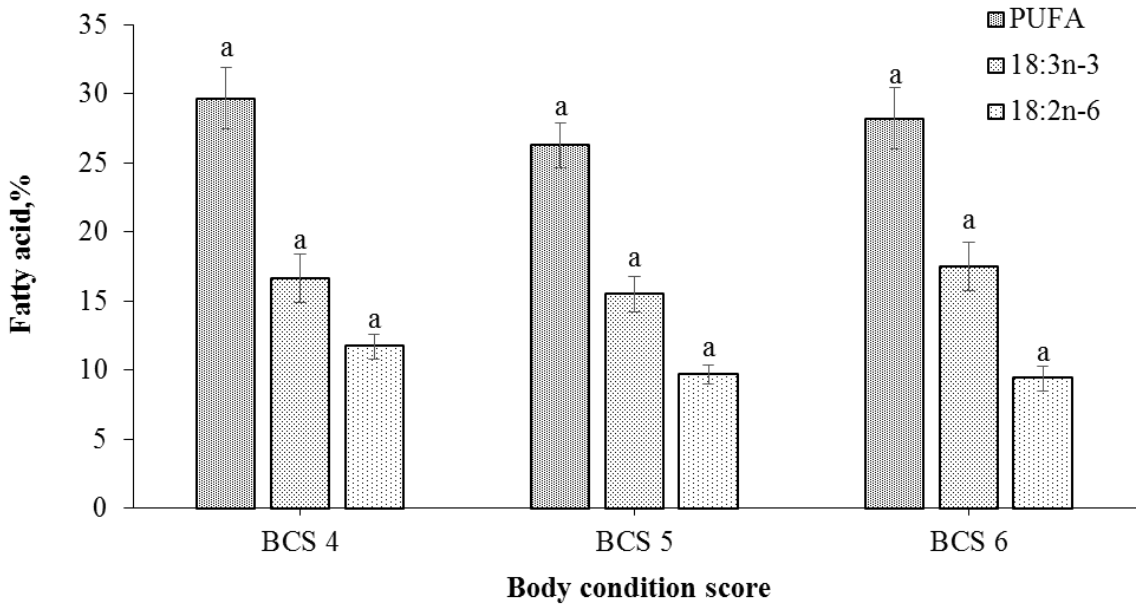

Figure 4.8 Percentage of total polyunsaturated fatty acids (PUFA), 18:2, and 18:3 FAs in intermuscular adipose tissue of horses in BCS 4 (n = 5), 5 (n = 9), and 6 (n = 5).

[abc]Within each category of FA, means without common letters differ ($P \leq 0.05$)

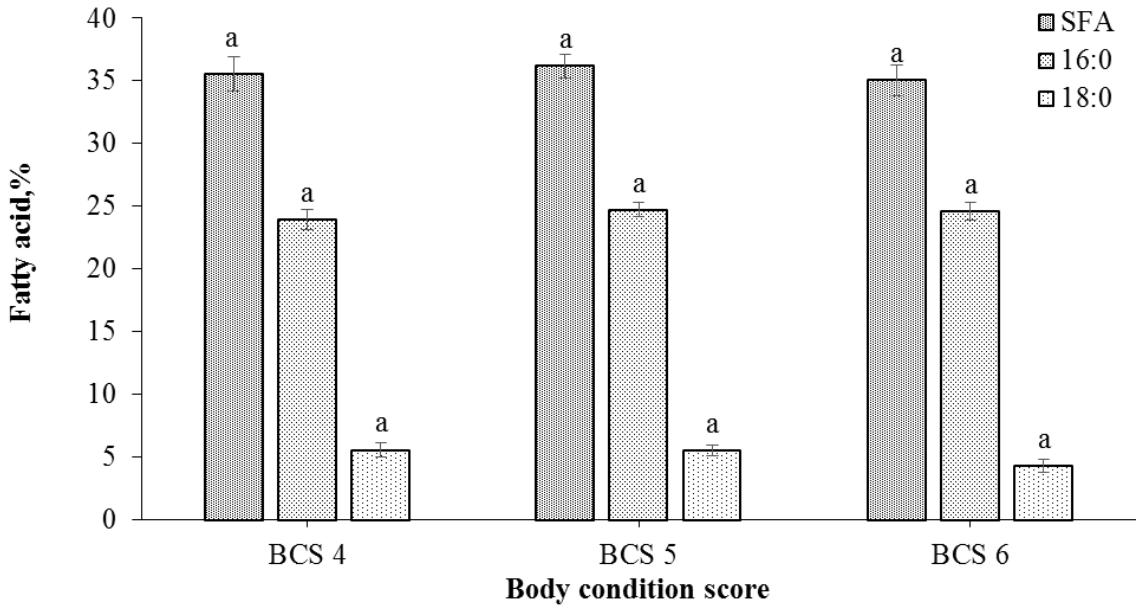

Figure 4.9 Percentage of total saturated fatty acid (SFA), 16:0, and 18:0 FAs in subcutaneous adipose tissue of horses in BCS 4 (n = 5), 5 (n = 9), and 6 (n = 5).

[abc]Within each category of FA, means without common letters differ ($P \leq 0.05$)

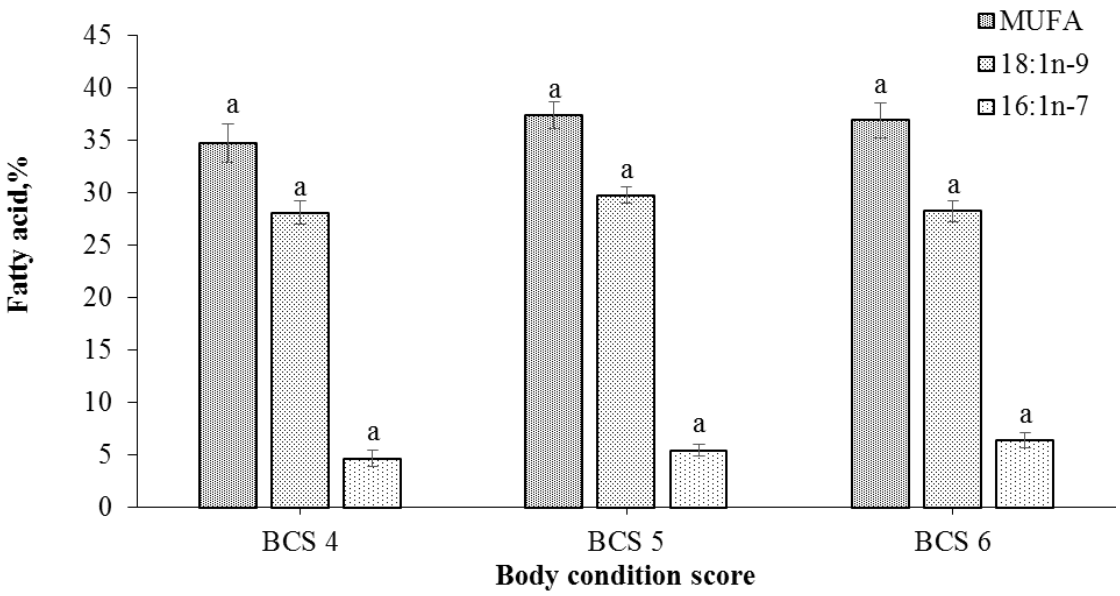

Figure 4.10 Percentage of total monounsaturated fatty acids (MUFA), 16:1, and 18:1 FAs in subcutaneous adipose tissue of horses in BCS 4 (n = 5), 5 (n = 9), and 6 (n = 5).

[abc]Within each category of FA, means without common letters differ ($P \leq 0.05$)

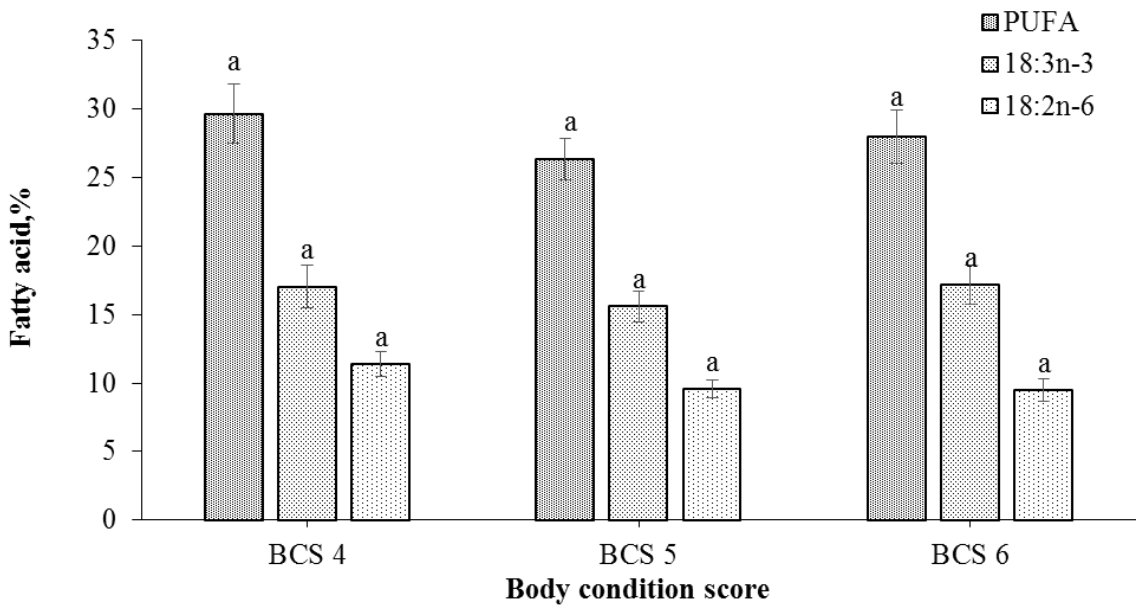

Figure 4.11 Percentage of total polyunsaturated fatty acids (PUFA), 18:2, and 18:3 FAs in subcutaneous adipose tissue of horses in BCS 4 (n = 5), 5 (n = 9), and 6 (n = 5).

[abc]Within each category of FA, means without common letters differ ($P \leq 0.05$)

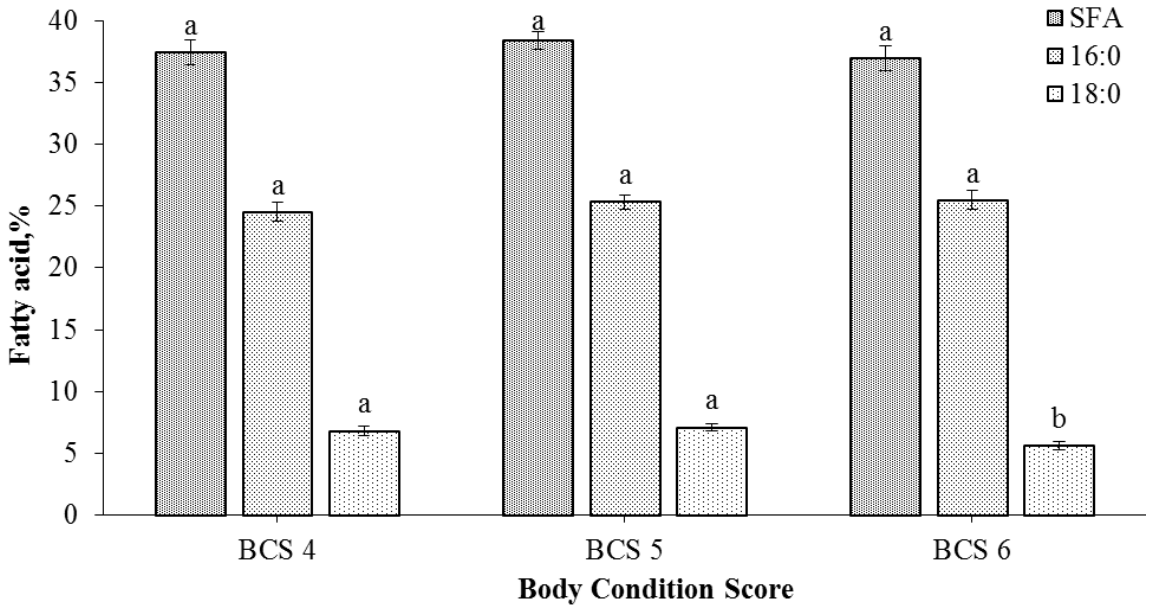

Figure 4.12 Percentage of total saturated fatty acid (SFA), 16:0, and 18:0 FAs in leaf fat of horses in BCS 4 (n = 5), 5 (n = 9), and 6 (n = 5).

[abc]Within each category of FA, means without common letters differ ($P \leq 0.05$)

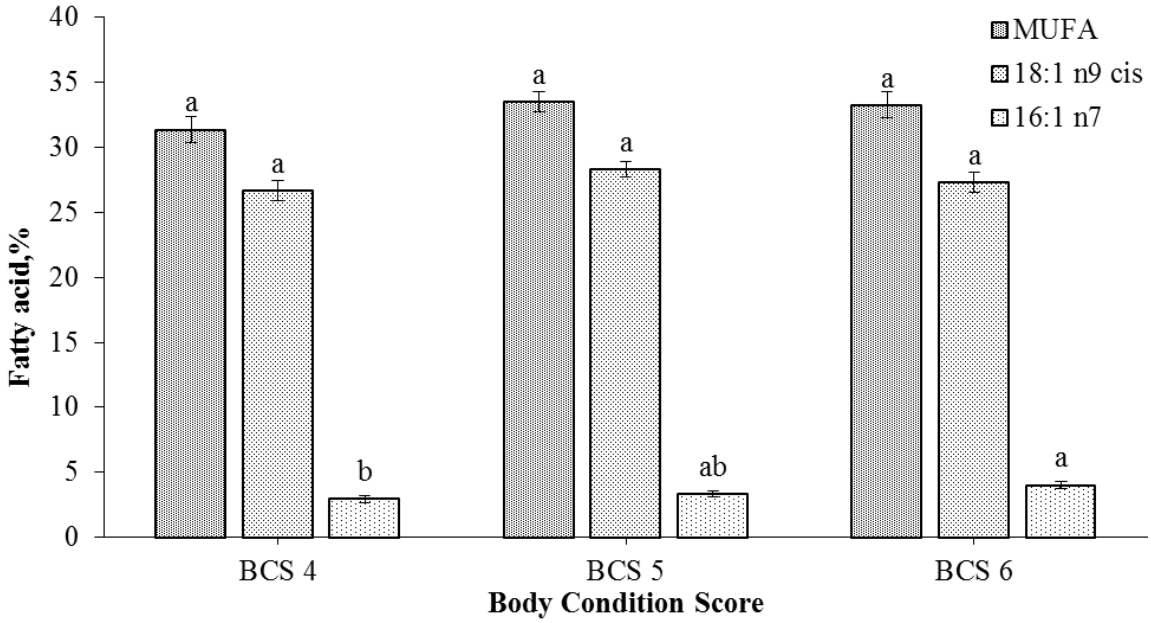

Figure 4.13 Percentage of total monounsaturated fatty acids (MUFA), 16:1, and 18:1 FAs in leaf fat of horses in BCS 4 (n = 5), 5 (n = 9), and 6 (n = 5).

[abc]Within each category of FA, means without common letters differ ($P \leq 0.05$)

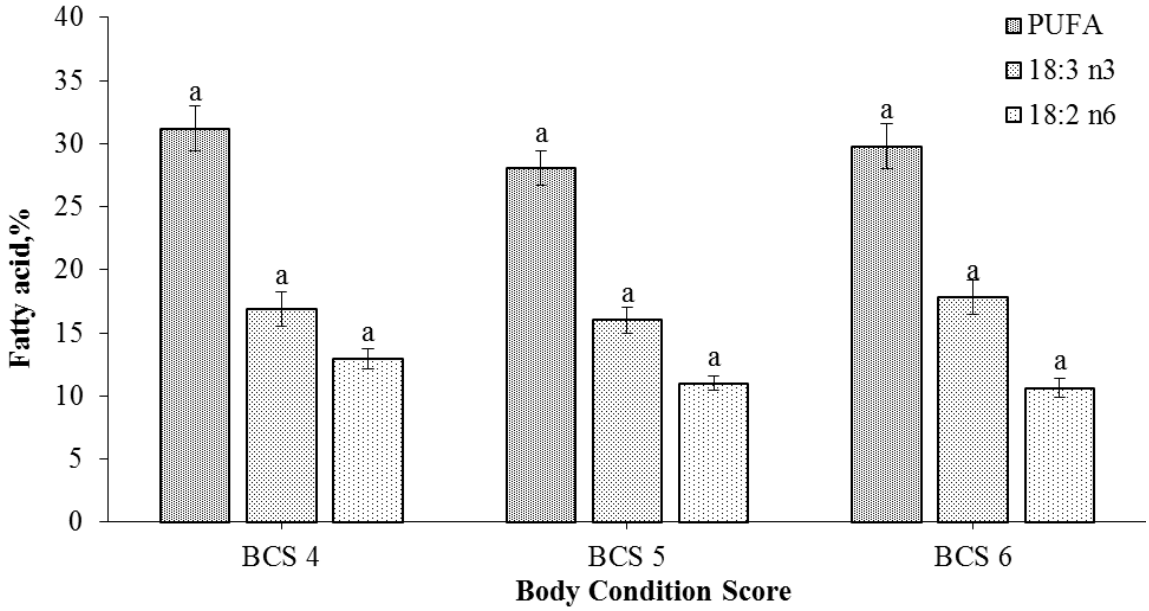

Figure 4.14 Percentage of total polyunsaturated fatty acids (PUFA), 18:2, and 18:3 FAs in leaf fat of horses in BCS 4 (n = 5), 5 (n = 9), and 6 (n = 5).

[abc]Within each category of FA, means without common letters differ ($P \leq 0.05$)

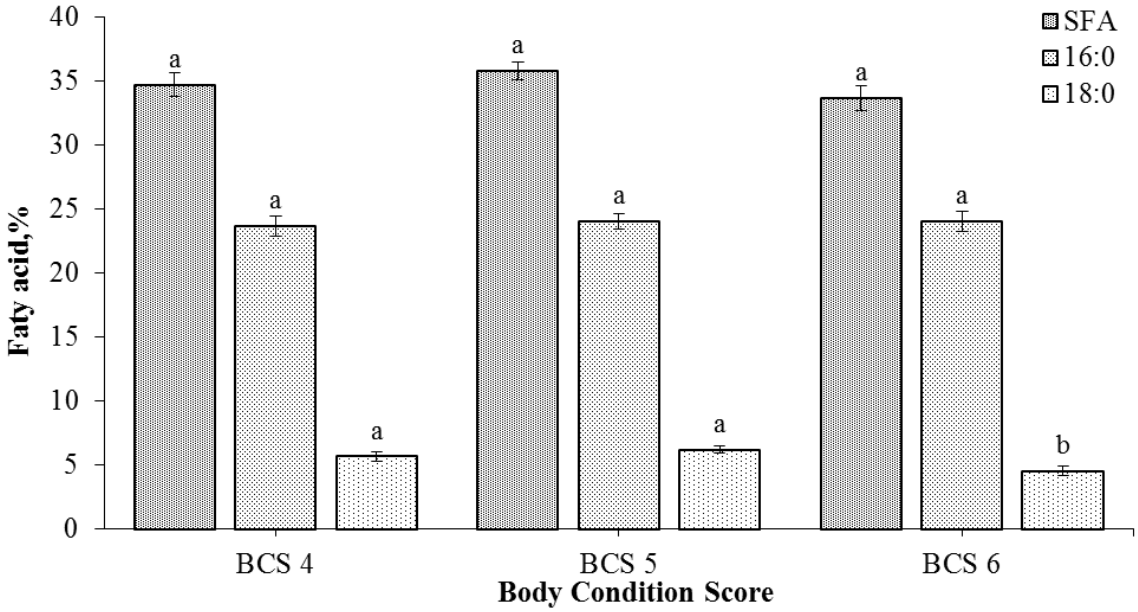

Figure 4.15 Percentage of total saturated fatty acid (SFA), 16:0, and 18:0 FAs in mesenteric adipose tissues of horses in BCS 4 (n = 5), 5 (n = 9), and 6 (n = 5).

[abc]Within each category of FA, means without common letters differ ($P \leq 0.05$)

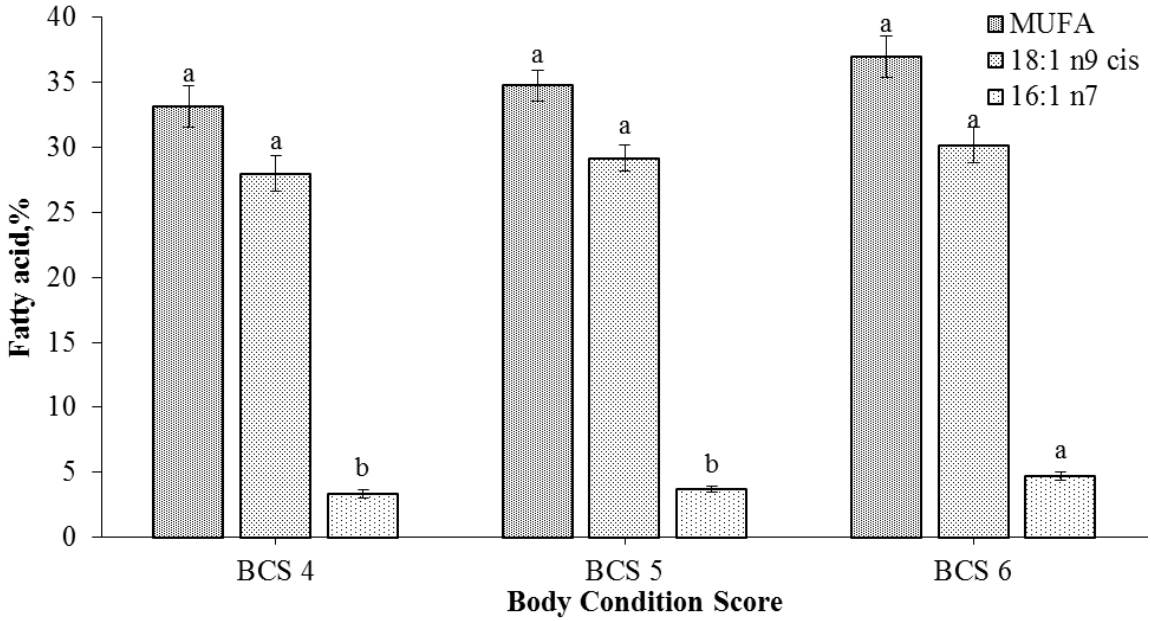

Figure 4.16 Percentage of total monounsaturated fatty acids (MUFA), 16:1, and 18:1 FAs in mesenteric adipose tissues of horses BCS 4 (n = 5), 5 (n = 9), and 6 (n = 5).

[abc]Within each category of FA, means without common letters differ ($P \leq 0.05$)

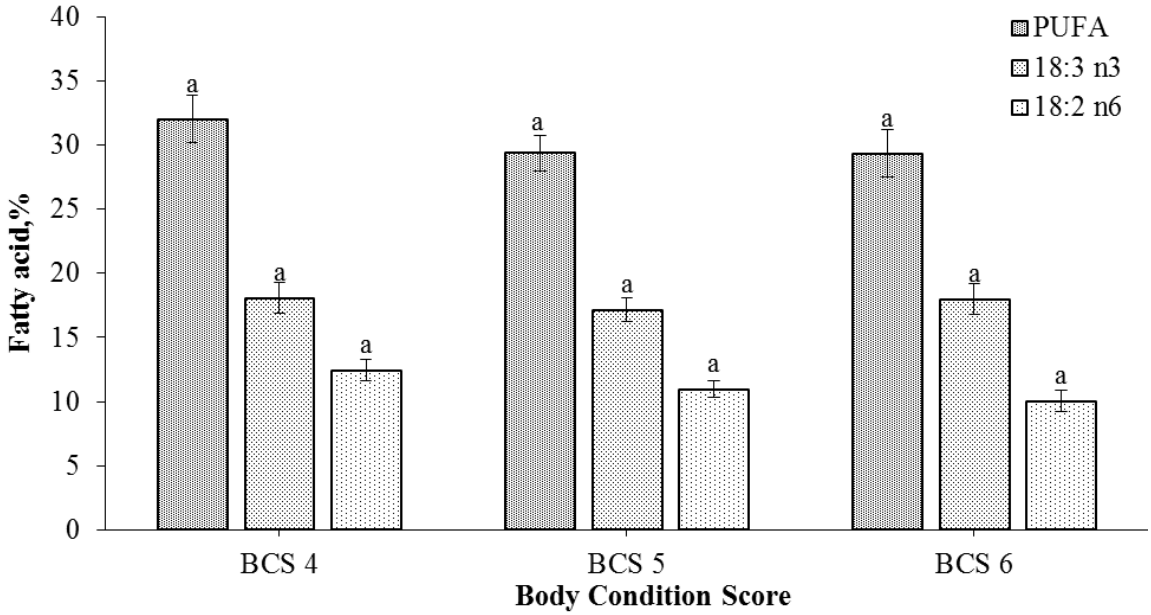

Figure 4.17 Percentage of total polyunsaturated fatty acids (PUFA), 18:2, and 18:3 FAs in mesenteric adipose tissues of horses in BCS 4 (n = 5), 5 (n = 9), and 6 (n = 5).

[abc]Within each category of FA, means without common letters differ ($P \leq 0.05$)

Table 4.1 Percentages of saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA), in cardiac adipose tissue depot of horses in BCS 4 (n = 5), 5 (n = 9), and 6 (n = 5)[1].

	Percentage, [2]%			
Fatty acid	BCS 4	BCS 5	BCS 6	*P* - value
SFA	36.56 ± 0.81	37.58 ± 0.6	36.29 ± 0.81	0.386
10:0	0.06 ± 0.01	0.05 ± 0	0.04 ± 0.01	0.108
12:0	0.22 ± 0.01	0.21 ± 0.01	0.21 ± 0.01	0.774
14:0	3.67 ± 0.18	3.45 ± 0.13	3.48 ± 0.18	0.588
15:0	0.39 ± 0.03	0.43 ± 0.02	0.45 ± 0.03	0.256
16:0	25.4 ± 0.87	26 ± 0.65	26.48 ± 0.87	0.684
17:0	0.64 ± 0.05	0.73 ± 0.03	0.62 ± 0.05	0.098
18:0	5.83 ± 0.39^{ab}	6.38 ± 0.29^{a}	4.69 ± 0.39^{b}	0.012
20:0	0.35 ± 0.02	0.32 ± 0.02	0.32 ± 0.02	0.515
MUFA	25.53 ± 1.3	27.98 ± 0.97	26.79 ± 1.3	0.334
14:1n-5	0.15 ± 0.01	0.13 ± 0.01	0.15 ± 0.01	0.432
16:1n-7	2.33 ± 0.27	2.45 ± 0.2	3.06 ± 0.27	0.141
17:1n-8	0.57 ± 0.03^{a}	0.68 ± 0.02^{b}	0.65 ± 0.03^{ab}	0.025
18:1n-9 cis	21.74 ± 1.09	23.98 ± 0.81	22.24 ± 1.09	0.225
20:1n-9	0.68 ± 0.04	0.7 ± 0.03	0.66 ± 0.04	0.660
22:1n-9	0.04 ± 0	0.03 ± 0	0.03 ± 0	0.060
PUFA	37.92 ± 1.82	34.44 ± 1.35	36.92 ± 1.82	0.286
18:2 trans	0.05 ± 0	0.05 ± 0	0.04 ± 0	0.394
18:2n-6	12.72 ± 0.58	13.11 ± 0.43	12.96 ± 0.58	0.869
18:3n-6	0.03 ± 0	0.03 ± 0	0.03 ± 0	0.684
18:3n-3	23.63 ± 1.39	19.88 ± 1.04	22.44 ± 1.39	0.105
20:2	0.44 ± 0.02	0.43 ± 0.02	0.41 ± 0.02	0.574
20:3n-6	0.06 ± 0.01	0.06 ± 0	0.06 ± 0.01	0.786
20:4n-6	0.9 ± 0.05	0.79 ± 0.04	0.89 ± 0.05	0.198
20:5n-3	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.957
22:6	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.766
SI	0.58 ± 0.02	0.6 ± 0.02	0.57 ± 0.02	0.306
P/S	1.04 ± 0.07	0.92 ± 0.05	1.02 ± 0.07	0.345

[a–c] Within each row of percentage categories, means without common letters differ (P ≤ 0.05).

[1]Values reported as least squares means ± SE.

[2]Fatty acid normalized percentages to total fatty acids.

Table 4.2 Percentages of saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA), in intermuscular adipose tissue depot of horses in BCS 4 (n = 5), 5 (n = 9), and 6 (n = 5)[1].

	Percentage, [2]%			
Fatty acid	BCS 4	BCS 5	BCS 6	*P* - value
SFA	35.78 ± 0.9	36.96 ± 0.67	34.5 ± 0.9	0.118
10:0	0.07 ± 0.01[a]	0.05 ± 0[b]	0.04 ± 0.01[b]	0.019
12:0	0.24 ± 0.02	0.21 ± 0.01	0.19 ± 0.02	0.152
14:0	4.53 ± 0.3	4.52 ± 0.23	4.45 ± 0.3	0.979
15:0	0.39 ± 0.02	0.44 ± 0.02	0.39 ± 0.02	0.151
16:0	24.23 ± 0.7	25.45 ± 0.52	24.82 ± 0.7	0.386
17:0	0.62 ± 0.05[ab]	0.68 ± 0.04[a]	0.49 ± 0.05[b]	0.015
18:0	5.45 ± 0.45[a]	5.36 ± 0.33[a]	3.88 ± 0.45[b]	0.035
20:0	0.27 ± 0.02	0.25 ± 0.01	0.23 ± 0.02	0.345
MUFA	34.56 ± 1.69	36.79 ± 1.26	37.3 ± 1.69	0.476
14:1n-5	0.36 ± 0.04	0.34 ± 0.03	0.42 ± 0.04	0.398
16:1n-7	4.45 ± 0.57[a]	5.14 ± 0.42[a]	6.45 ± 0.57[b]	0.065
17:1n-8	0.82 ± 0.04[a]	0.98 ± 0.03[b]	0.88 ± 0.04[a]	0.009
18:1n-9 cis	28.08 ± 1.23	29.52 ± 0.92	28.7 ± 1.23	0.636
20:1n-9	0.81 ± 0.07	0.77 ± 0.05	0.82 ± 0.07	0.827
22:1n-9	0.05 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.217
PUFA	29.65 ± 2.24	26.26 ± 1.67	28.2 ± 2.24	0.474
18:2 trans	0.04 ± 0[a]	0.04 ± 0[a]	0.03 ± 0[b]	0.046
18:2n-6	11.71 ± 0.9	9.68 ± 0.67	9.4 ± 0.9	0.154
18:3n-6	0.03 ± 0.01	0.03 ± 0	0.03 ± 0.01	0.572
18:3n-3	16.62 ± 1.73	15.47 ± 1.29	17.48 ± 1.73	0.642
20:2	0.42 ± 0.04	0.35 ± 0.03	0.41 ± 0.04	0.271
20:3n-6	0.05 ± 0.01	0.04 ± 0	0.05 ± 0.01	0.219
20:4n-6	0.68 ± 0.06	0.58 ± 0.04	0.73 ± 0.06	0.144
20:5n-3	0.04 ± 0.01	0.03 ± 0	0.05 ± 0.01	0.056
22:6	0.04 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.117
SI	0.56 ± 0.02	0.59 ± 0.02	0.53 ± 0.02	0.128
PS	0.83 ± 0.08	0.72 ± 0.06	0.82 ± 0.08	0.422

[a–c] Within each row of percentage categories, means without common letters differ ($P \leq 0.05$).

[1]Values reported as least squares means ± SE.

[2]Fatty acid normalized percentages to total fatty acids.

Table 4.3 Percentages of saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA), in subcutaneous adipose tissue depot of horses in BCS 4 (n = 5), 5 (n = 9), and 6 (n = 5)[1].

	Percentage, [2]%			
Fatty acid	BCS 4	BCS 5	BCS 6	P value
SFA	35.54 ± 1.38	36.19 ± 0.97	35.05 ± 1.23	0.766
10:0	0.06 ± 0[a]	0.05 ± 0[ab]	0.04 ± 0[b]	0.044
12:0	0.23 ± 0.02	0.2 ± 0.01	0.2 ± 0.02	0.296
14:0	4.45 ± 0.35	4.35 ± 0.25	4.73 ± 0.31	0.652
15:0	0.41 ± 0.03	0.43 ± 0.02	0.41 ± 0.03	0.674
16:0	23.92 ± 0.81	24.73 ± 0.57	24.58 ± 0.72	0.712
17:0	0.64 ± 0.06	0.68 ± 0.04	0.52 ± 0.05	0.105
18:0	5.59 ± 0.6	5.51 ± 0.43	4.32 ± 0.54	0.202
20:0	0.25 ± 0.02	0.24 ± 0.01	0.24 ± 0.02	0.898
MUFA	34.78 ± 1.83	37.46 ± 1.29	36.95 ± 1.64	0.498
14:1n-5	0.36 ± 0.06	0.38 ± 0.04	0.46 ± 0.05	0.348
16:1n-7	4.72 ± 0.77	5.48 ± 0.54	6.41 ± 0.69	0.281
17:1n-8	0.87 ± 0.07	1.04 ± 0.05	0.89 ± 0.06	0.106
18:1n-9 cis	28.1 ± 1.12	29.78 ± 0.79	28.31 ± 1	0.376
20:1n-9	0.71 ± 0.07	0.75 ± 0.05	0.83 ± 0.06	0.395
22:1n-9	0.03 ± 0.01	0.03 ± 0	0.04 ± 0.01	0.339
PUFA	29.68 ± 2.17	26.36 ± 1.53	28.01 ± 1.94	0.464
18:2 trans	0.05 ± 0.01	0.05 ± 0	0.04 ± 0.01	0.321
18:2n-6	11.37 ± 0.93	9.58 ± 0.66	9.49 ± 0.83	0.265
18:3n-6	0.04 ± 0	0.03 ± 0	0.03 ± 0	0.173
18:3n-3	17.03 ± 1.57	15.61 ± 1.11	17.18 ± 1.4	0.621
20:2	0.43 ± 0.04	0.38 ± 0.03	0.42 ± 0.04	0.559
20:3n-6	0.05 ± 0.01	0.05 ± 0	0.05 ± 0.01	0.697
20:4n-6	0.66 ± 0.06	0.6 ± 0.04	0.73 ± 0.05	0.188
20:5n-3	0.04 ± 0	0.03 ± 0	0.03 ± 0	0.391
22:6	0.03 ± 0.01	0.03 ± 0	0.03 ± 0	0.660
SI	0.55 ± 0.03	0.57 ± 0.02	0.54 ± 0.03	0.714
P/S	0.84 ± 0.08	0.74 ± 0.06	0.8 ± 0.07	0.627

[a–c] Within each row of percentage categories, means without common letters differ ($P \leq 0.05$).

[1]Values reported as least squares means ± SE.

[2]Fatty acid normalized percentages to total fatty acids.

Table 4.4 Percentages of saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA), in leaf fat of horses in BCS 4 (n = 5), 5 (n = 9), and 6 (n = 5)[1].

	Percentage, [2]%			
Fatty acid	BCS 4	BCS 5	BCS 6	*P* value
SFA	37.44 ± 0.98	38.39 ± 0.73	36.96 ± 0.98	0.487
10:0	0.07 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.302
12:0	0.25 ± 0.01	0.21 ± 0.01	0.2 ± 0.01	0.095
14:0	4.35 ± 0.23	4.18 ± 0.17	4.27 ± 0.23	0.826
15:0	0.4 ± 0.02	0.44 ± 0.02	0.41 ± 0.02	0.441
16:0	24.49 ± 0.77	25.31 ± 0.57	25.46 ± 0.77	0.623
17:0	0.74 ± 0.05[a]	0.81 ± 0.03[ab]	0.65 ± 0.05[b]	0.032
18:0	6.81 ± 0.37[a]	7.1 ± 0.27[a]	5.63 ± 0.37[b]	0.017
20:0	0.32 ± 0.02	0.3 ± 0.02	0.29 ± 0.02	0.685
MUFA	31.37 ± 1.01	33.53 ± 0.75	33.26 ± 1.01	0.241
14:1n-5	0.22 ± 0.02	0.2 ± 0.02	0.24 ± 0.02	0.400
16:1n-7	2.94 ± 0.29[a]	3.3 ± 0.22[ab]	4.02 ± 0.29[b]	0.050
17:1n-8	0.72 ± 0.04[a]	0.88 ± 0.03[ab]	0.81 ± 0.04[b]	0.028
18:1n-9 cis	26.68 ± 0.79	28.31 ± 0.59	27.29 ± 0.79	0.254
20:1n-9	0.78 ± 0.07	0.8 ± 0.05	0.85 ± 0.07	0.787
22:1n-9	0.03 ± 0	0.03 ± 0	0.04 ± 0	0.185
PUFA	31.19 ± 1.81	28.08 ± 1.35	29.79 ± 1.81	0.392
18:2 trans	0.05 ± 0.01	0.05 ± 0	0.04 ± 0.01	0.280
18:2n-6	12.93 ± 0.78	10.98 ± 0.58	10.64 ± 0.78	0.099
18:3n-6	0.03 ± 0	0.03 ± 0	0.03 ± 0	0.657
18:3n-3	16.89 ± 1.38	16.02 ± 1.03	17.83 ± 1.38	0.583
20:2	0.49 ± 0.03	0.38 ± 0.03	0.41 ± 0.03	0.073
20:3n-6	0.04 ± 0	0.04 ± 0	0.04 ± 0	0.530
20:4n-6	0.71 ± 0.05	0.61 ± 0.03	0.73 ± 0.05	0.085
20:5n-3	0.03 ± 0	0.03 ± 0	0.03 ± 0	0.901
22:6	0.02 ± 0	0.02 ± 0	0.02 ± 0	0.374
SI	0.60 ± 0.03	0.63 ± 0.02	0.59 ± 0.03	0.487
P/S	0.83 ± 0.06	0.74 ± 0.05	0.81 ± 0.06	0.492

[a–c] Within each row of percentage categories, means without common letters differ (P ≤ 0.05).

[1]Values reported as least squares means ± SE.

[2]Fatty acid normalized percentages to total fatty acids.

Table 4.5 Percentages of saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA), in mesenteric adipose tissue of horses in BCS 4 (n = 5), 5 (n = 9), and 6 (n = 5)[1].

	Percentage, [2]%			
Fatty acid	BCS 4	BCS 5	BCS 6	*P* value
SFA	34.75 ± 0.94	35.82 ± 0.7	33.67 ± 0.94	0.214
10:0	0.07 ± 0.01	0.33 ± 0.01	0.03 ± 0.01	0.071
12:0	0.09 ± 0.02	0.71 ± 0.01	0.05 ± 0.02	0.090
14:0	0.58 ± 0.24	0.57 ± 0.18	0.69 ± 0.24	0.584
15:0	0.8 ± 0.03	0.9 ± 0.02	0.64 ± 0.03	0.799
16:0	0.92 ± 0.8	0.72 ± 0.6	0.72 ± 0.8	0.920
17:0	0.08 ± 0.05	0.59 ± 0.03	0.12 ± 0.05	0.082
18:0	0.01 ± 0.34[a]	0.24 ± 0.26[a]	0.04 ± 0.34[b]	0.006
20:0	0.52 ± 0.02	0.62 ± 0.02	0.27 ± 0.02	0.523
MUFA	33.19 ± 1.6	34.77 ± 1.19	36.97 ± 1.6	0.271
14:1n-5	0.76 ± 0.03	0.8 ± 0.02	0.48 ± 0.03	0.762
16:1n-7	0.03 ± 0.33[b]	0.45 ± 0.25[b]	0.01 ± 0.33[a]	0.027
17:1n-8	0.31 ± 0.05	0.15 ± 0.04	0.22 ± 0.05	0.305
18:1n-9 cis	0.54 ± 1.37	0.5 ± 1.02	0.28 ± 1.37	0.544
20:1n-9	0.39 ± 0.07	0.98 ± 0.05	0.26 ± 0.07	0.385
22:1n-9	0.2 ± 0.02	0.84 ± 0.02	0.17 ± 0.02	0.196
PUFA	33.06 ± 1.84	29.41 ± 1.37	29.36 ± 1.84	0.477
18:2 trans	0.25 ± 0.01	0.57 ± 0.01	0.33 ± 0.01	0.254
18:2n-6	0.14 ± 0.83	0.18 ± 0.62	0.05 ± 0.83	0.142
18:3n-6	0.28 ± 0	0.41 ± 0	0.12 ± 0	0.277
18:3n-3	0.78 ± 1.24	0.55 ± 0.93	0.96 ± 1.24	0.777
20:2	0.41 ± 0.05	0.28 ± 0.04	0.21 ± 0.05	0.405
20:3n-6	0.14 ± 0.01	0.06 ± 0	0.11 ± 0.01	0.139
20:4n-6	0.14 ± 0.05	0.07 ± 0.04	0.76 ± 0.05	0.136
20:5n-3	0.26 ± 0	0.11 ± 0	0.33 ± 0	0.259
22:6	0.07 ± 0	0.03 ± 0	0.05 ± 0	0.067
SI	0.53 ± 0.02	0.56 ± 0.02	0.51 ± 0.02	0.192
PS	0.92 ± 0.07	0.83 ± 0.05	0.88 ± 0.07	0.553

[a–c] Within each row of percentage categories, means without common letters differ ($P \leq 0.05$).

[1]Values reported as least squares means ± SE.

[2]Fatty acid normalized percentages to total fatty acids.

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